# STUDY OF HLA DR AND DQ TYPES IN PATIENTS WITH PEMPHIGUS VULGARIS AS COMPARED TO THAT IN HEALTHY SUBJECTS


**DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE RULES AND REGULATIONS FOR THE M.D. BRANCH XX DERMATOLOGY, VENEREOLOGY AND LEPROSY EXAMINATION OF THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY TO BE HELD IN APRIL, 2015**

# CERTIFICATE

This is to certify that the dissertation entitled **"Study of HLA DR and DQ types in patients with pemphigus vulgaris as compared to that in healthy subjects"** is the bonafide original work of **Dr. Anuradha Priyadarshini.**

This study was undertaken at the **Christian Medical College and Hospital, Vellore** from November 2013 to August 2014, under my guidance and direct supervision, in partial fulfilment of the requirement for the award of the **MD degree (Branch XX) in Dermatology, Venereology and Leprosy of the Tamil Nadu Dr. M.G.R. Medical University**.

**Guide & Head of the Department**

**Dr.Renu George**

Professor and Head,
Department of Dermatology Venereology and Leprosy
Christian Medical College,
Vellore,
Tamil Nadu .

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**Principal**

**Dr. Alfred Job Daniel**

Christian Medical College,
Vellore,
Tamil Nadu 632004

# DECLARATION

I hereby declare that this M.D. dissertation entitled "**Study of HLA DR and DQ types in patients with pemphigus vulgaris as compared to that in healthy subjects**" is a bonafide work done by me, Dr.Anuradha Priyadarshini, under the guidance of Dr.Renu George, Professor, Department of Dermatology, Venereology and Leprosy, Christian Medical College, Vellore.

This work has not been submitted to any other university in part or full.

Dr.Anuradha Priyadarshini,
Postgraduate registrar,
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# ACKNOWLEDGEMENTS

I express my deepest gratitude to my teacher and guide Dr.Renu George for her guidance, encouragement, support and invaluable advice at every step of this study. Without her dedicated involvement this dissertation would not have been possible.

I would like to thank Dr.Dharshini, Dr.Poonam Jalan, Dr.Lydia Mathew and Dr.Sivaranjini, Assistant Professors, Dept. Of Dermatology Unit I, for their support and their invaluable and timely inputs.

I am extremely grateful to all the patients who participated in this study.

I thank Dr.Dolly Daniel for helping me with the HLA typing for this study, Mrs.Shanthi and all the other staff at HLA laboratory for their selfless help in doing the HLA typing for this study.

I thank Dr.Jayakanthan for his cooperation and help in doing the serum IgG4 titres.

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Finally, I owe my thanks to my husband Dr.Ajit Agarwal for his tireless personal and professional support. And my family for the support and encouragement they have always given me.

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January 27, 2014

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Sub: **Fluid Research grant project**:
To study the HLA DR and DQ types in patients with pemphigus as compared to that in healthy subjects.
Dr. Anuradha Priyadarshini, Dermatology, Venereology and Leprosy Unit I, Dr. Renu George, Dermatology, Venereology and Leprosy, Dr. Dolly Daniel, Transfusion medicine and Immunohaematology, Dr. Jayakanthan Kabeer Doss, Rheumatology and Clinical Immunology, Dr. Visalakshi Jeyaseelan, Biostatistics, Dr. Santosh Varughese, Nephrology.

Ref: IRB Min. No. 8549 [OBSERVE] dated 12.11.2013

Dear Dr. Anuradha Priyadarshini,

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Nihal Thomas, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Nihal Thomas
Secretary (Ethics Committee)
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Dr. NIHAL THOMAS
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Cc: Dr. Renu George, Dermatology, Venereology and Leprosy, CMC



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Ref: IRB Min. No. 8549 [OBSERVE] dated 12.11.2013

Dear Dr. Anuradha Priyadarshini,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project entitled "To study the HLA DR and DQ types in patients with pemphigus as compared to that in healthy subjects." on November 12th 2013.

The Committees reviewed the following documents:

1. IRB application format
2. Curriculum Vitae' Dr. Anuradha Priyadarshini, Dr. Renu George, Dr. Dolly Daniel, Dr. Jayakanthan Kabeer Doss, Dr. Visalakshi Jeyaseelan, Dr. Santosh Varughese.
3. Informed Consent form (English, Tamil, Bengali & Hindi)
4. Patient Information Sheet (English, Tamil, Bengali & Hindi)



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**Dr. Nihal Thomas,**
MD., MNAMS., DNB (Endo), FRACP (Endo), FRCP (Glas) (EDIN)
Deputy Chairperson
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Additional Vice Principal (Research)


5. Proforma
6. No of documents 1-5

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on November 12th 2013 in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632002.

| Name | Qualification | Designation | Other Affiliations |
|---|---|---|---|
| Dr. Simon Rajaratnam | MBBS, MD, DNB (Endo), MNAMS (Endo), PhD (Endo), FRACP | Professor, Endocrinology, CMCH. | Internal, Clinician |
| Dr. T. Balamugesh | MBBS, MD(Int Med), DM, FCCP (USA) | Professor, Pulmonary Medicine, CMCH. | Internal, Clinician |
| Dr. Chandra Singh | MS, MCH, DMB | Professor, Urology, CMCH. | Internal, Clinician |
| Dr. Visalakshi | MPH, PhD | Lecturer, Dept. of Biostatistics, CMC. | Internal, Statistician |
| Dr. Benjamin Perakath | MBBS, MS, FRCS | Professor, Colorectal Surgery, CMCH. | Internal, Clinician |
| Dr. Anup Ramachandran | Ph. D | The Wellcome Trust Research Laboratory Gastrointestinal Sciences, CMCH. | Internal, Basic Medical Scientist |
| Dr. Mathew Joseph | MBBS, MCH | Professor, Neurosurgery, CMCH. | Internal, Clinician |




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| Mrs. Pattabiraman | B. Sc, DSSA | Social Worker, Vellore | External, Lay person |
| Mr. C. Sampath | B. Sc, BL | Legal Expert, Vellore | External, Legal Expert |
| Rev. Joseph Devaraj | B. Sc, BD | Chaplaincy Department, CMCH. | Internal, Social Scientist |
| Dr. Vathsala Sadan | M.Sc, PhD | Professor, Community Health Nursing, CMCH. | Internal, Nurse |
| Dr. Ebenezer Ellen Benjamin | M.Sc, PhD | Professor, Maternity Nursing, CMCH. | Internal, Nurse |
| Dr. B. J. Prashantham | MA(Counseling Psychology), MA(Theology), Dr. Min (Clinical Counselling) | Chairperson, Ethics Committee, IRB. Director, Christian Counseling Centre, Vellore | External, Social Scientist |
| Dr. Anuradha Rose | MBBS, MD | Assistant Professor, Community Health, CMCH. | Internal, Clinician |
| Dr. Jayaprakash Muliyil | B. Sc, MBBS, MD, MPH, Dr PH (Epid), DMHC | Retired Professor, Vellore | External, Scientist & Epidemiologist |
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We approve the project to be conducted as presented.

The Institutional Ethics Committee expects to be informed about the progress of the project, any **adverse events** occurring in the course of the project, any **amendments in the protocol and the patient information / informed consent**. On completion of the study you are expected to submit a copy of the **final report**. Respective forms can be downloaded from the following link: http://172.16.11.136/Research/IRB_Polices.html in the CMC Intranet and in the CMC website link address: http://www.cmch-vellore.edu/static/research/Index.html.

*Fluid Grant Allocation:*

*A sum of 50,000 INR (Rupees Fifty Thousand only) will be granted for 1 year.*

Yours sincerely

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

Dr. NIHAL THOMAS
MD.,MNAMS.,DNB(Endo),FRACP(Endo),FRCP(Edin),FRCP(Glasg)
SECRETARY - (ETHICS COMMITTEE)
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Cc: Dr. Renu George, Dermatology, Venereology and Leprosy, CMC

IRB Min. No. 8549 [OBSERVE] dated 12.11.2013



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# BAGAYAM, VELLORE 632002, TAMIL NADU, INDIA

Ref: FG/8549/11/2013 February 19, 2014

The Treasurer
Christian Medical College,
Vellore.

Dear Mr. Denzil,

Sub: **Fluid Research grant project**:
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Dr. Anuradha Priyadarshini, Dermatology, Venerology and Leprosy Unit I,
Dr. Renu George, Dermatology, Venerology and Leprosy, Dr. Dolly Daniel, Transfusion medicine and Immunohematology, Dr. Jayakanthan Kabeer Doss, Rheumatology and Clinical Immunology, Dr. Visalakshi Jeyaseelan, Biostatistics, Dr. Santosh Varughese, Nephrology.

Ref: IRB Min. No. 8549 dated 12.11.2013

The Institutional Review Board at its meeting held on November 12th 2013 vide IRB Min. No. **8549** accepted the project *for a total sum 50,000 INR (Rupees Fifty Thousand only) will be granted for 1 year. If overspent the excess should be debited form the respective departmental or Special funds.* Kindly arrange to transfer the sanctioned amount to a separate account to be operated by Dr. Anuradha Priyadarshini and Dr. Renu George.

Yours sincerely,

Dr. Nihal Thomas
Secretary (Ethics Committee)
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Dr. NIHAL THOMAS
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22Y261. RF. Dr. Anuradha Priyadarshini - Derm-I (8549)

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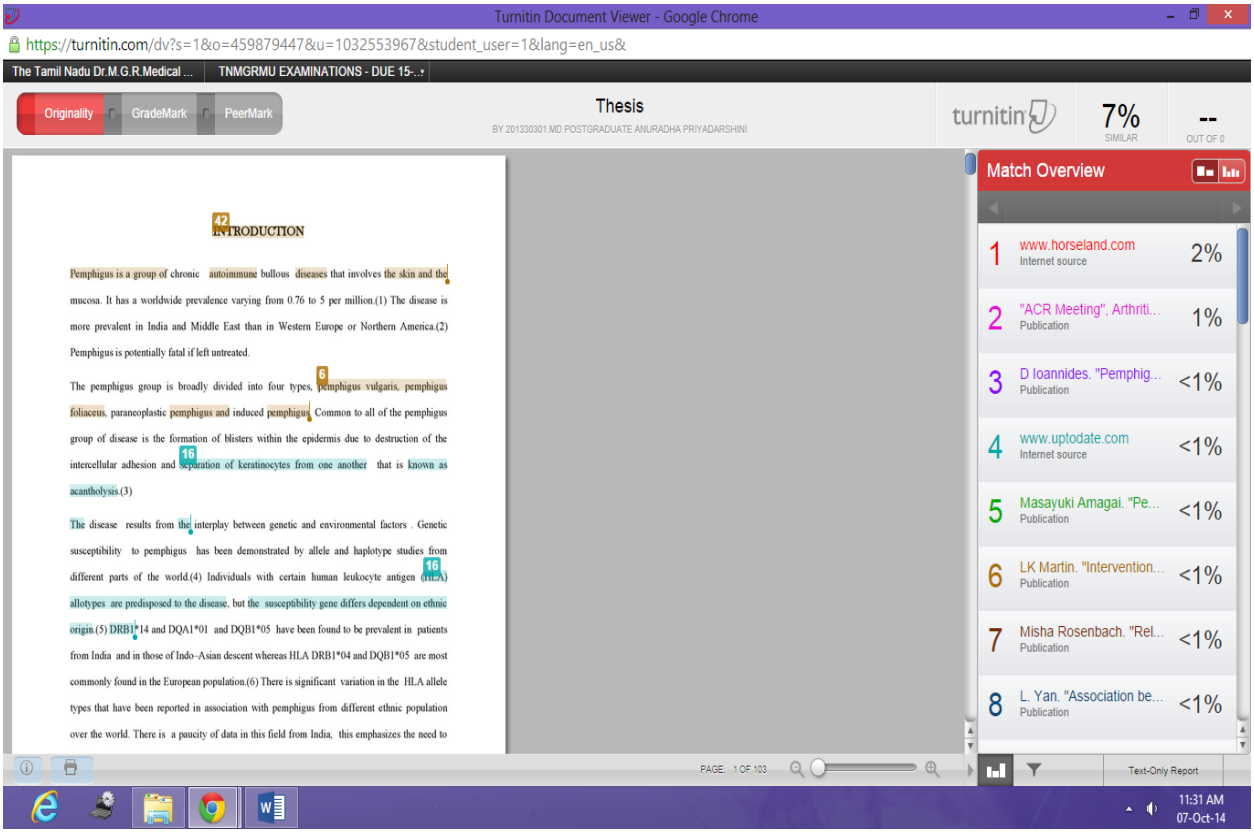

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ABSIS – Autoimmune Bullous Skin Disorder Intensity Score

ANA – Antinuclear antibodies

Dsg – Desmoglein

DIF – Direct Immunofluorescence

ELISA – Enzyme Linked Immunosorbent Assay

HLA – Human Leukocyte Antigen

IgG4 – Immunoglobulin G4

IIF – Indirect Immunofluorescence

IVIG – Intravenous immunoglobulins

M:F – Male : Female

MHC – Major Histocompatibility Complex

MMF – Mycophenolate mofetil

PDAI – Pemphigus Disease Area Index

PCR SSOP – Polymerase Chain Reaction – Sequence specific oligonucleotide probe

# ABSTRACT

TITLE: Study of HLA DR and DQ types in patients with pemphigus vulgaris as compared to that in healthy subjects.

DEPARTMENT: Dept. of Dermatology Venereology and Leprosy, Christian Medical College, Vellore.

NAME OF THE CANDIDATE : Dr.Anuradha Priyadarshini

DEGREE AND SUBJECT: MD DERMATOLOGY, VENEREOLOGY AND LEPROSY.

NAME OF THE GUIDE: Dr.Renu George, Prof and Head, Dept. of Dermatology, Venereology and Leprosy, Christian Medical College, Vellore.

**Objectives**: Pemphigus is an acquired immunobullous disease of the skin and mucosa with genetic predisposition and autoimmune aetiology. HLA Class II genes are associated with pemphigus. IgG1 and IgG4 antibodies are the pathogenic antibodies in pemphigus of which IgG4 is associated with active disease. The primary objective of this study was to find out the HLA DR and DQ types in pemphigus vulgaris in India, and to compare it to that in normal healthy controls. Secondary objective was to look at the correlation between disease severity in terms of PDAI score and serum IgG4 levels.

**Methods**: A hospital based cross-sectional study done over a period of 10 months. The diagnosis of pemphigus was established by clinical examination, biopsy, DIF and anti-Dsg ELISA. Pemphigus vulgaris patients were enrolled for HLA DR and DQ typing by PCR-SSOP method. Renal transplant donors were taken as controls. Clinical examination and PDAI score was done in pemphigus vulgaris and foliaceus patients with active disease and it was correlated with serum total IgG4 levels in them. Chi-square test was applied to analyze the frequencies of HLA DR and

DQ types in cases versus controls. Spearman Rho was used to correlate serum IgG4 levels and PDAI scores. Mann-Whitney U test was used to look for the association between HLA types and disease severity.

**Results**: The study included 72 patients, 54 (75%) pemphigus vulgaris and 18 (25%) pemphigus foliaceus. Mean age at disease onset for pemphigus vulgaris patients was 39.2 ±13.3 years, M:F ratio 1:1.45. In pemphigus foliaceus group mean age at disease onset was 44.3 ± 12.9 years, M:F ratio 3.5:1. The mean PDAI score was 23.5 in pemphigus vulgaris and 33.8 in foliaceus patients. Typical features on histopathology was seen in 88.4% pemphigus vulgaris cases and 100% foliaceus cases. DIF was positive in 91.4% vulgaris and 100% foliaceus cases.

HLA typing was done in 50 patients and compared with 50 controls. HLA DRB1*14 was present in 94% cases versus 36% controls ($p<0.001$). HLA DQB1*05 was present in 94% cases versus 48% controls ($p<0.001$). The haplotype DRB1*14, DQB1*05 was present in 88% cases versus 28% controls ($p<0.001$). Negative association was found with DRB1*15 (14% cases versus 32% controls, $p<0.05$) and DQB1*06 (16% cases versus 38% controls, $p<0.05$).

HLA DQB1*03 was associated with more severe disease.

Mean serum IgG4 was 1114.4mg/L in pemphigus vulgaris patients and 1147.1mg/L in foliaceus patients. Positive correlation between PDAI and IgG4 levels was present in pemphigus vulgaris patients, but did not reach significance levels. This correlation was not observed in pemphigus foliaceus group.

**Key words:** Pemphigus, PDAI, HLA DRB1, HLA DQB1, IgG4.

# INTRODUCTION

Pemphigus is a group of chronic autoimmune bullous diseases that involves the skin and the mucosa. It has a worldwide prevalence varying from 0.76 to 5 per million.(1) The disease is more prevalent in India and Middle East than in Western Europe or North America.(2) Pemphigus is potentially fatal if left untreated.

The pemphigus group is broadly divided into four types, pemphigus vulgaris, pemphigus foliaceus, paraneoplastic pemphigus and induced pemphigus. Common to all of the pemphigus group of disease is the formation of blisters within the epidermis due to destruction of the intercellular adhesion and separation of keratinocytes from one another that is known as acantholysis.(3)

The disease results from the interplay between genetic and environmental factors. Genetic susceptibility to pemphigus has been demonstrated by allele and haplotype studies from different parts of the world.(4) Individuals with certain human leukocyte antigen (HLA) allotypes are predisposed to the disease, but the susceptibility gene differs dependent on ethnic origin.(5) DRB1*14 and DQA1*01 and DQB1*05 have been found to be prevalent in patients from India and in those of Indo–Asian descent whereas HLA DRB1*04 and DQB1*05 are most commonly found in the European population.(6) There is significant variation in the HLA allele types that have been reported in association with pemphigus from different ethnic population around the world. There is a paucity of data in this field from India, this emphasizes the need to further study the HLA types in pemphigus patients.

Autoimmunity in pemphigus is characterized by the presence of antibodies to the intercellular adhesion molecules and other target antigens. Circulating and skin-fixed antibodies are present in all types of pemphigus. Clinical and experimental evidence suggests the pathogenic role of these antibodies in blister formation.(7) Studies have clearly demonstrated

that the autoantibodies in pemphigus patients mainly belong to the IgG1 and IgG4 subclasses.(7–11) IgG4 subtype is mainly thought to mediate acantholysis. In patients with active pemphigus vulgaris, IgG4 autoantibodies against desmogleins were found to predominate.

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# AIMS AND OBJECTIVES

**Primary objective:** To study the prevalence of HLA DR and DQ types in patients with pemphigus vulgaris and compare it to that in healthy individuals without pemphigus.

**Secondary objective:** To correlate the severity of disease in pemphigus vulgaris and pemphigus foliaceus patients having active disease as measured by Pemphigus Disease Area Index (PDAI) score with the serum levels of total IgG4.

# REVIEW OF LITERATURE

Pemphigus refers to a group of autoimmune blistering diseases involving the skin and mucous membranes. It is characterized histologically by intraepidermal bulla formation due to acantholysis and immunopathologically by the presence of in vivo bound and circulating immunoglobulins that are directed against the cell surface antigens and adhesion molecules on keratinocytes. The disease is potentially fatal if left untreated.

**History**

The word pemphigus is derived from the greek word "pemphix" which means bubble.(1) Walter Lever in his monograph gave an account of the first description of the disease. According to Lever the first description of pemphigus in two patients was given by MacBride in 1777. Pemphigus foliaceus was identified as a distinct type by Cazenave in 1844. The classical description of acantholysis (loss of cell-cell adhesion) by Civatte, in 1943, led to the unambiguous differentiation of pemphigus from other subepidermal blistering diseases. The modern history of pemphigus began in 1964 with the demonstration of circulating antibodies to keratinocyte surface antigens by Beutner and Jordan. The discovery of these antibodies led to the understanding of autoimmune pathogenesis of pemphigus. Subsequently the antigenic targets were identified to be desmosomal cadherins. Desmoglein 1 (Dsg1) in pemphigus foliaceus and desmoglein 3 (Dsg3) protein in pemphigus vulgaris were eventually identified by immunohistochemistry.(12)

**Epidemiology**

The worldwide prevalence of pemphigus varies from 0.76 to 5 cases per million per year and depends on the geographic area and ethnicity under study. A higher incidence of about 16 to

32 cases per million per year is seen in the Jewish race.(1) People of Mediterranean descent and of the Middle East are also known to have a higher prevalence as compared to the Western population.(3) An average yearly increase of 11% was seen in a population based cohort study from UK. Younger age at onset is seen in India and Middle East.(2) The age at presentation of disease from other parts of the world is the fifth decade.(13) Children are rarely affected except in areas of endemic pemphigus foliaceus.

Pemphigus is known to affect males and females equally. However a recent internet-based survey of patients found a female predominance.(14)

**Indian scenario**

In India the prevalence of pemphigus has been reported between 0.9 to 1.8% in dermatology outpatient attendees.(15,16) An incidence of 4.4 cases per million was found on a clinic-based questionnaire survey in Thrisur, India.(17) Over fifty percent of pemphigus patients were under the age of 40 years at presentation.(18) Contrasting results have been seen with regard to gender ratio from Indian studies with reports of male preponderance of 3:1 to female preponderance of 1.2 :1.(15,18) ***Table 1*** shows epidemiological data on pemphigus from different countries.

## Table 1- Epidemiological data on pemphigus from different countries

| Study | Region | Duration of study | Number of patients | Age at onset in years | Male :Female ratio |
|---|---|---|---|---|---|
| Kanwar A J et al. (15) | North India | 1988-2004 | 328 | 39 | 1:1.2 |
| Kandan et al(19) | South India | 2006-2008 | 65 | 44.6 | 1:1.8 |
| Kim MR et al. (20) | Korea | 1993-2008 | 199 | 46.1 | 1: 1.1 |
| Chams-Davatchi et al. (21) | Iran | 1984-2003 | 1209 | 42 | 1:1.5 |
| Ljubojevic et al. (22) | Croatia | 1980-1998 | 159 | 53 | 2:1 |
| Gupta VK et al. (14) | Various[a] | 2011[b] | 171 | 51.8 | 1:2.25 |

[a] North America, Canada, Mexico. [b]questionnaire based study, data collected via internet.

## Etiopathogenesis

### *Role of HLA antigens*

There is convincing evidence to support the genetic basis for susceptibility to pemphigus. Pemphigus is an example of polygenic disease that does not result from the Mendelian inheritance of single gene but from the interaction of several different genes as explained by the concept of epistasis.(4) Since pemphigus is known to be an autoimmune disease due to loss of immune tolerance, the candidate genes studied widely are the MHC class II genes which are important in regulating immunity. The association of MHC class II genes with pemphigus has been reported for over 25 years.(4)

Major Histocompatibility Complex (MHC) or Human leukocyte antigens (HLA) as they are called in humans, span 4000 kbps on the short arm of chromosome 6p21. The MHC consists of a linked set of genetic loci that encode many proteins that are involved in presenting antigens to the T-cells. They encode two distinct classes of cell surface molecules: class I and class II. The class I molecules are expressed on the surfaces of virtually all nucleated cells at varying densities, while class II molecules are more restricted to cells of the immune system, primarily B lymphocytes and monocytes, and their expression on other cells is increased on stimulation by inflammatory cytokines. There are three different class I molecules namely HLA A, B, and C and three class II molecules DR, DQ, and DP. Other genes that map at the MHC locus include those that encode a few complement components, TNF-alpha and lymphotoxin. These are called the MHC class III genes.(23)

The HLA genes exhibit a high degree of polymorphism. The polymorphism for the Class II genes occurs due to change in nucleotide sequences coding for alpha-1 and beta -1 domains.

As a result of the polymorphism for a gene, variants of that gene are produced which are called alleles. The number of these alleles can be varied and over 280 alleles are known for the HLA DRB gene and 46 alleles are known for the DQB gene.(24) Since the alleles are expressed in high frequency it is unlikely that the two alleles occupying the locus on homologous chromosomes in an individual will be the same. The particular combination of alleles present on a HLA locus is called a haplotype. The expression of HLA alleles is co-dominant. Codominance is the relation between two alleles of a gene wherein neither one is recessive, therefore the presence of one allele does not mask the expression of the other allele and both alleles of gene are expressed.(25) Polymorphism contributes to the wide diversity of HLA genes within individuals and populations. The polymorphism of HLA genes is of paramount importance in their function in presenting antigen.

Class II molecules present antigens to T-cells and a T-cell that recognizes an antigen bound to one type of HLA will not recognize the same antigen when bound to another HLA allele. This specificity of antigen presentation and recognition is referred to as the MHC restriction of T-cells.(26,27) The HLA Class II molecule contains two transmembrane chains that are the alpha and beta chains (one for each locus) and each chain has two domain. The two distal domains form the peptide binding pocket by a non-covalent association between them. The different substitutions of amino acids particularly in this peptide binding pocket determines the variation of HLA alleles.(23) It is the variation in this peptide binding pocket that is an important determinant of antigen presentation and therefore responsible for the immunogenesis in pemphigus.(28)

The difference between the peptide binding pocket of disease-associated HLA DR and DQ types in comparison with their normal types explains the structural basis by which specific HLA types confer susceptibility to pemphigus. The presence of negatively charged amino acids in the beta-chain at the peptide binding site is responsible for the binding of desmogleins that are the main target antigens of pemphigus. This is in contrast to the molecules from the non-disease associated HLA gene sequences where the amino acids at the peptide binding site are positively charged. The negative charge of the peptide binding site confers selective binding to the desmoglein peptides which are positively charged. Thus amino acids that form the peptide binding residue are critical for the presentation of peptides and they are determined by the mutagenesis of DRB1*0402 and DQ B1*0503.(28)

Several conventional case-control studies have consistently identified DR4 and DR14alleles in association with pemphigus vulgaris in most parts of the world and DQB1 and DQA1 alleles in certain other specific regions. The association of these different alleles is seen with different frequencies in different ethnicities. The susceptibility genes of HLA DR4, DR14 and DQ types have also been found in pemphigus folicaeus, but as opposed to pemphigus vulgaris, no single allele is over expressed in the foliaceus patients.(29)

Over 95% pemphigus patients have HLA DR4 or DR6 or both.(4) Of the DRB1 alleles nine subtypes (DRB1*0402, *0403, *0406, *1401, *1404, *1405, *1408, *0804,*0802) have been associated with pemphigus vulgaris as reported from various studies over different population groups.(5)(30–33) ***(Table 2)*** Of these DRB1 *0402 is more common in the European and American population while *1404 is more common in the Indo-Asian pemphigus patients. DQB1*0302 and DQB1*0503 of the DQB alleles are also reported

worldwide in pemphigus vulgaris patients. DQB1*0503 is more often associated with Indo-Asian pemphigus patients while DQB1*0302 is more frequent in European population.(6) Four alleles at the DQA1 locus, DQA1*0101, *0104, *0401 and *0301 have been seen in pemphigus vulgaris of which the DQA1*0101 has also been found in pemphigus patients from India.(34) Negative correlation has been postulated with a handful alleles such as the DQB1*02 and *06, DRB1*03, *13 and *17, DQA1*0103. Although no preventive benefit has been demonstrated with the possession of these alleles.(5,34)

## Table 2- HLA types reported worldwide with pemphigus vulgaris

| Region/country | No of patients | HLA DR types | HLA DQB types | HLA DQA types |
|---|---|---|---|---|
| Asia | | | | |
| Indian(34) | 37 | 1404, 0402 | 0503, 0302 | 0101 |
| Chinese(35) | 27 | 0403,0406,1401,1404,1405 | 0503, 0302 | |
| Japanese(36–38) | 51, 32, 16 | 0406, 0802, 1401, 1405, 1408 | 0503, 0302 | 0104,0301 |
| Pakistani(39) | 19 | 1404 | 0503 | 0101 |
| Middle East | | | | |
| Iranian(40) | 20 | 0402, | 0302 | 0301 |
| Sardinian(41) | 16 | 0402, 1401, | 0503, 0302 | 0104, 0301 |
| Europe | | | | |
| Jewish(33) | 26 | 0402, 1401, | 0302 | - |
| Italian (42) | 87 | 0402, 0804, 1401, 1404 | 0503, 0302 | 0104, 0301, 0401 |
| French (43) | 37 | 0402, 1401, 1404 | 0503, 0302 | - |
| South American | | | | |
| Spanish(44) | 11 | 0402, 1401, | 0503, 0302 | |
| Argentinian(45) | 47 | 0402,1401, | 0503, 0302 | - |

HLA DR B1*0402 is the most frequently associated allele in Ashkenazi Jews and Non-Jewish Western population. Other alleles such as the DRB1*1401 and 1404 are reported from French, Italian, Japanese population. A meta-analysis of 18 case control studies representing

Asian, Caucasian and other nationalities indicated that DRB1*04, DRB1*08 and DRB1*14 were associated with significant risk of pemphigus vulgaris. The meta-analysis did not include DR6 alleles. As there was considerable heterogeneity in the DRB1*04 observed, ethnicity stratified test was done which showed reduced heterogeneity for Asians. DRB1*08 had an increased significance only for the Caucasian group. A protective role was suggested by the negative correlation reported with DRB1*03, DRB1*07 and DRB1*15.(30)

Of the DR6 alleles DQB1*0302 is consistently elevated in Jewish pemphigus vulgaris patients. DQB1*0503 is the other more widely associated allele of the DQB1 type in non-Jewish population.(5)

The DQA alleles that are associated with pemphigus vulgaris patients include DQ A1* 0104 and DQ A1*0301. DQA1*0104 has been found in increased frequencies in Japanese pemphigus patients, while DQA1*0101 has been associated with pemphigus patients from India, Pakistan and Europe.(5,34,39,41)

**HLA allele types in Indian patients**

Wilson et al's study published in 1994 included 50 patients of pemphigus from India and 20 patients from UK. They reported HLA DR1 and DR4 were increased in pemphigus patients of both countries. DR1 (DRB1*01) was seen in 26% cases versus 14% controls among Indian patients and 26% cases versus 10% controls among the patients from UK. DR4 DRB1*04 was seen in 34% cases versus 26% controls in Indian patients, and in 47% cases versus 34% controls in patients from UK. They also reported a negative correlation of DR2 (DRB1*15 or DRB1*16) in both the groups. Although the data regarding DR6 was not

presented, they mentioned it to be slightly higher in male patients as compared to female patients.(46)

Delgado et al reported HLA allele frequencies and haplotype analysis of 39 pemphigus patients from India.(34) The study constituted of 2 subset of patients, one from New Delhi and the other from Ahmedabad. The HLA alleles of the patient group was compared to haplotypes of healthy relatives. In this study they found HLA DRB1*1404, DRB3*0202, DQA1*0101, DQB1*0503 as the most prevalent alleles in patients as compared to that in controls. In addition to these they also identified a significantly higher prevalence for HLA DQB1*0302 among patients from Ahmedabad only. Interestingly, HLA DRB1*0402 which has been the most frequently established association with pemphigus worldwide was found only occasionally in patients in this study and in association with DQB1*0302 that is similar to the haplotypes identified in Jewish population. The study also identified DQB1*02 to be more prevalent in normal relatives than in the patient group, thereby identifying it's presence to have a protective effect for the development of pemphigus.(34)

Saha et al's study has laid further emphasis on the ethnic difference between pemphigus patients of the Indo-Asian population and the Western Europeans in the expression of HLA types.(6) The study included 96 Western-European patients and 57 Indo-Asian patients. The HLA type DRB1*1404 was the most significantly overexpressed allele in the Indo-Asian population as compared to the Western-European, although DRB1*0402 was also reported from the Indo-Asians. The other alleles found to be significantly overexpressed were the DQ B1*0503 and DQB1*0302. Since DQB1*0503 and DQB1*0302 are frequently seen in association with DR14 and DR4 respectively they speculated that this association may be due to linkage disequilibrium and not a true predisposing factor for disease.(6)

Haplotype studies and sequence analysis of peptides of the predominant HLA types of pemphigus patients have been done to identify disease relevant alleles. The studies identified DRB1*0402 and DQB1*0503 to be specific types that confer susceptibility to disease by their unique peptide pockets that can present desmoglein antigens. Whereas the other alleles such as DRB1*1401 and DQB1*0302 are overexpressed probably due to linkage disequilibrium.(5)

Other genes of the Major Histocompatibility Complex which have been studied for their involvement in pemphigus include the Class III genes that encode Tumor necrosis factor-alpha and lymphotoxin-alpha (LTA). Polymorphisms in TNF-alpha promoter gene is associated with susceptibility with pemphigus.(47)

***Role of non-HLA genes***

Non–HLA genetic targets have also been explored in the causation of pemphigus. Pemphigus is characterized by autoantibodies of the IgG1 and IgG4 subclass with the latter being relevant in active stage of the disease.(48) Therefore the genes involved in type switching of the immunoglobulin gamma chain is a potential candidate gene for pemphigus. Various Th2 cytokines are involved in the antibody production and inflammation. Of these cytokines IL6 gene polymorphism is the only one that showed significant difference in patients with pemphigus.(49)

A recent study based on structural analysis of the anti-Dsg antibodies suggested that the antibody response is autoantigen derived.(48) This prompted investigators to find out whether variations within locus of desmogein 3 and desmoglein 1 genes confer susceptibility

in pemphigus vulgaris and foliaceus respectively. The studies following this line of investigation found certain polymorphism markers for the desmoglein 1 and desmoglein 3 genes in pemphigus foliaceus and pemphigus vulgaris respectively. But in both of these instances it was associated with the presence of HLA alleles that were known to confer susceptibility to pemphigus thus suggesting that they may have additive effects.(4,50)

***Role of Autoantibodies***

Autoantibodies of the IgG isotype are found in tissue and the serum of patients with pemphigus. The primary role of anti-desmoglein antibodies in the pathophysiology of pemphigus is well established.(51) Evidence for this comes from in vitro and in vivo observations. Neonates born to mothers with active pemphigus develop transient pemphigus-like disease due to transplacental transfer of antibodies. Pemphigus-like phenotype is induced in neonatal mice by the passive transfer of IgGs from patients with pemphigus.(52)

The binding of antibodies to their targets i.e. desmogleins leads to acantholysis. This may be directly due to steric hindrance (direct hit theory) or indirectly by proteasomes and signal transduction. The ultrastructural studies of the antibodies demonstrated that pathogenic IgG antibodies bind specifically to particular ectodomains on the desmogleins at their amino terminal. The binding of the antibodies to desmogleins results in disassembly of the desmosomes. The antigen – antibody complex are internalized thereby depleting the desmosomes at the intercellular space.(48,53)

The anti-desmoglein antibodies in the serum can be detected either by indirect immunofluorescence (IIF) or enzyme-linked immunosorbent assays (ELISA). Specific ELISA using recombinant pemphigus antigens was developed by Ishii et al.(54) In their

study ELISA detected anti-Dsg3 antibodies in 46 /49 pemphigus vulgaris patients and anti-Dsg1 antibodies in 44/46 pemphigus foliaceus patients. None of the 53 controls showed positivity for the antibodies in this study. Thus they concluded the test to be sensitive and specific diagnostic tool for pemphigus. Amagai et al found 97.9% of pemphigus vulgaris patients had positivity for Dsg3 ELISA and 97.5% of pemphigus foliaceus patients has positivity for Dsg1 ELISA.(55) They also found that the Dsg antibody levels paralleled the course of disease. They proposed the Dsg ELISA be included as diagnostic criteria for pemphigus since the presence of anti-Dsg1 and absence of anti-Dsg3 correlated with the pemphigus foliaceus, likewise presence of anti-Dsg3 and absence of anti-Dsg1 correlated with mucosal pemphigus, and both antibodies were present in mucocutaneous pemphigus. This finding was also supported by Schmidt et al (study on 71 vulgaris and 48 foliaceus patients) and Daneshpazhooh et al (study on 73 pemphigus vulgaris patients).(56,57)

Indian studies however did not concur with these results. The Dsg antibody levels did not correlate with the disease phenotype. Khandpur et al in their study of 66 pemphigus patients and 77 controls found that anti-Dsg1 and anti-Dsg3 antibody levels were elevated in both pemphigus vulgaris and foliaceus patients.(58) Anand et al reported that anti-Dsg3 titres were elevated in patients with cutaneous pemphigus and conversely anti-Dsg1 titres were elevated in patients of mucosal pemphigus vulgaris, therefore the antibody titres did not predict the clinical phenotype of pemphigus.(59)

The anti-desmoglein antibody levels correlate with severity of disease. Kumar et al found that the anti-dsg3 and anti-dsg1 antibody levels determined the severity of oral and skin lesions in pemphigus patients. Their results was based on study of 44 pemphigus patients that included 38 vulgaris and 6 foliaceus patients.(60)

The IgG antibodies have been classified into four subclasses of antibodies designated G1, G2, G3 and G4 based in order of their normal serum concentrations. The subclasses differ in their heavy chains. IgG4 is the least common IgG in healthy individuals. It is also the most complex of them. Due to its unique structure it can act as a blocking antibody but does not form large immune complexes. It is not associated with complement activation.(61)

The predominant antibody subclass seen in pemphigus are the IgG1 and IgG4.(8,9,62,63) Bhol et al, in a case control study done by them on pemphigus (study on 21 patients with active disease, 18 patients in remission and 22 healthy controls), described the presence of IgG1 and IgG4 in high concentrations against a specific peptide of the desmogleinin active disease, and IgG1 antibodies specific to different peptide in patients in remission and the controls. This study highlighted the differences between the two subclasses of IgG antibodies in terms of epitope specificity and pathogenicity. The same group, using immunoblot technique, also demonstrated IgG1 and IgG4 antibodies in pemphigus patients with active disease while only IgG1 in patients in remission.(48) Several studies done thereafter have supported the pathogenic role of IgG4 antibodies. IgG4 is found to be the predominant antibody in active phase of the disease while IgG1 is seen in patients even during remission. The role of IgG subclasses in pemphigus was studied in a case control study model comparing the antibodies in patients to that in first-degree relatives of patients and in healthy unrelated individual. This study showed the presence of IgG4 in 62% of patients as compared to 6% in first degree relatives and was absent in healthy controls.(62)

A case was reported of pemphigus vulgaris occurring in a neonate, born to a mother known to have pemphigus. The antibody subclasses as tested by indirect immunofluorescence and ELISA revealed the presence of higher titres of IgG4 in both the mother and the neonate as

compared to the negative test for IgG1.(64) This report illustrates the capacity of IgG4 antibodies to induce acantholysis and blisters. Experimental studies done on mice with injection of IgG4 anitbodies developed against desmoglein3 produced acantholytic blisters on the mice skin.(52)

IgG4 antibodies are probably responsible for the initiation of active disease. This is suggested by the observations that were made in endemic pemphigus patients. In the regions endemic for pemphigus foliaceus normal individuals were found to have IgG1 antibodies. IgG1 was the predominant antibody in patients prior to onset of clinical disease. Whereas IgG4 antibody titres were high once the disease manifest clinically.(65) Dhandha et al in their study on immunoglobulin subtypes in pemphigus patients emphasized the role of IgG4. They found that IgG4 was the sole subclass of antibody that correlated with clinical phenotype. The IgG4 anti-desmoglein 3 antibody was elevated in mucous predominant and mucocutaneous pemphigus and G4 anti-desmoglein 1 antibody was elevated in the cutaneous pemphigus, while this difference was not significant for other IgG isotypes. They also reported a significant positive correlation between disease duration and anti-Dsg3 IgG4 antibody levels, an increase in the antibody levels was associated with longer disease duration. In this study it was also found that patients who were carriers of HLA gene types known to be associated with pemphigus had higher IgG4 antibodies that those patients negative for the HLA types.(63)

***Role of environmental factors***

Environmental factors play a vital role in the pathogenesis of endemic pemphigus. The presence of the nigrimanum simulium fly and other hematogenous insects in high frequency is associated with endemic pemphigus.(66) Several recent studies have implicated the role of

other environmental factors such as ingestion of certain food, contact with pesticides in the pathogenesis of pemphigus vulgaris. Foods and beverages such as garlic, berries, red wine and tea that are high in thiols, phenol and tannin compounds respectively may trigger the flares of disease activity. Occupational exposure to pesticides of the organophosphate group may induce pemphigus by inhibiting cholinergic receptors at the keratinocyte.(67) Hormonal factors have been implicated as a trigger for pemphigus, this is based on reports of pemphigus during pregnancy. Significantly higher oral contraceptive pills usage has been associated with pemphigus, although the mechanism by which it acts as trigger for pemphigus is still not known. Cigarette smoking on the other hand is said to have a protective role. This is explained by the immunosuppression caused by chronic nicotine exposure.(67) Drugs are a known causative factor for induced pemphigus. Drugs containing thiol group such as penicillamine and captopril can provoke blistering directly by their biochemical interference with cell-cell adhesion molecules, activating plasminogen and by altering the desmoglein antigenic structure and thus inducing antibody formation. On the other hand phenol containing drugs such as rifampicin and cephalosporins lead to release of interleukin and tumor necrosis factor from keratinocyte. These cytokines further lead to activation of plasminogen activators contributing to acantholysis.(68)

***Pathophysiology of blister formation in pemphigus***

Desmosomes are intercellular bridges that attach two adjacent keratinocyte cell membranes. Desmosomes are composed of three groups of proteins namely (i) desmosomal cadherins, (ii) plakins and (iii) armadillo family proteins (plakophilins and plakoglobins). Of the cadherins the desmogleins are the main target in pemphigus.(69) Antibodies induce acantholysis by directly affecting desmoglein function. They interfere with cis-trans interactions (steric hindrance) and bring about conformational change in the desmoglein ectodomain.

Antibodies indirectly inhibit desmoglein function by activating kinases such as the protein kinase C, plasminogen activator and p38 mitogen activated protein kinase (p38MAPK) that lead to desmoglein disassembly.(70)

The basal cell shrinkage theory that has been put forth to explain acantholysis in pemphigus describes cytoskeletal collapse and keratinocyte cell volume reduction as the primary event in loss of adhesion of keratinocytes. The basal cell shrinkage may also be due to presence of antibodies to acetylcholine receptor on keratinocytes.(71) Antibodies to several keratinocyte proteins have been found in pemphigus. Grando et al postulated that acantholysis is the end result of action of all these antibodies thus naming it the multiple hit theory.(72) The antibodies to acetylcholine receptor affects normal function of keratinocytes leading to rounding up of cells and loss of desmogleins. This is followed by stimulation of apoptotic pathways and therefore the mechanism of blister formation has also been termed apoptolysis.(73)

The clinical feature and differentiation of different types of pemphigus is determined by the distribution of desmogleins in epidermis and relative concentrations of specific antibodies to them as explained by the desmoglein compensation theory.(3) The theory is based on the assumption that antibodies to one isoform of desmoglein will inactivate only that specific isoform of desmoglein. Desmoglein 1 is present throughout the epidermis of skin and weakly expressed in mucosa. Whereas desmoglein 3 is present in lower part of epidermis and strongly expressed in the entire mucosal epithelium. In pemphigus foliaceus antibodies are present against Dsg 1 thus blisters develop in the superficial layers of epidermis while the loss of Dsg1 in lower epidermis is compensated for by Dsg3. Similarly the mucosa is not affected because of the strong expression of desmoglein 3. On the other hand in case of

mucosal predominant pemphigus antibodies present against desmoglein 3 destroy it leading to erosions in oral mucosa. Mucosal erosions are the main manifestation in this situation since dsg1 is not adequately expressed in mucosa to compensate for the loss of Dsg3. The skin is not affected since Dsg 1 compensates for the loss of Dsg3 in lower epidermis. In mucocutaneous type of pemphigus antibodies against desmoglein 1 are also present which explains the development of blisters on the skin.(29)

***Classification of pemphigus***

The pemphigus diseases are classified based on clinical features, the level of the split within the epidermis and the antibody isotype that is predominantly seen on immunofluorescence studies. ***(Table 3)***

## Table 3- Classification of pemphigus (29)

| Type of pemphigus | Immunoglobulin type | Antigenic target |
|---|---|---|
| Pemphigus vulgaris | | |
| Clinical Subtypes - | | |
| Mucosal dominant | IgG | Dsg 3 |
| Mucocutaneous | IgG | Dsg 1 + Dsg 3 |
| Variant - | | |
| Pemphigus vegetans | IgG | Dsg 1 + Dsg3 |
| Pemphigus foliaceus | IgG | Dsg 1 |
| Variant | | |

| | | |
|---|---|---|
| Pemphigus erythematosus | IgG | Dsg 1 |
| Pemphigus herpetiformis | IgG | Dsg 1 |
| Induced pemphigus | IgG | Heterogeneous |
| Paraneoplastic pemphigus | IgG | Dsg3, Dsg1, (Dsc), Plectin, desmoplakin, BP230, envoplakin, periplakin, |
| IgA pemphigus | | |
| Subtypes- | | |
| SCPD type | IgA | Desmocollin 1 |
| IEN type | IgA | ?unknown |
| IgA pemphigus vulgaris | IgA | Dsg 3 |
| IgA pemphigus foliaceus | IgA | Dsg 1 |
| Endemic pemphigus | | |
| Subtypes – | | |
| Brazilian | IgG | Dsg1, (Dsg3, ?Desmocollin1-3) |
| Tunisian | IgG | Dsg 1, Dsg 3 |
| Colombian | IgG | Dsg 1 + α |

IEN – Intraepidermal neutrophilic IgA type, SCPD – subcorneal pustular dermatosis type

**Clinical features of pemphigus vulgaris and foliaceus**

In most countries pemphigus vulgaris is more common than pemphigus foliaceus. The percentage of pemphigus vulgaris varies between 70 to 92percent of all cases. A few exceptions to this is seen in Tunisia and Finland where the ratio of pemphigus foliaceus is twice as common as pemphigus vulgaris.(3)

Pemphigus vulgaris is the most common type of pemphigus seen in India. The percentage of pemphigus vulgaris as reported by Indian studies ranges from 70% to 89% and that of pemphigus foliaceus from 3% to 20%.(18) Pemphigus vegetans, pemphigus erythematosus and paraneoplastic pemphigus are seen occasionally and together constitute about 5% of the pemphigus cases. It is a disease of 5$^{th}$ decade, however, in India over 50% of affected patients are below 40 years of age. Slight female predominance has been reported with M:F ratio of 0.8:1, 1:1.48, and 1:1.82.(18,19,74)

***Pemphigus vulgaris and its variants***

Patients with pemphigus vulgaris present with mucosal erosions in 50 to 70% patients. (75) The disease may be confined to oral cavity in mucosal predominant subtype of pemphigus vulgaris. The oral lesions are often painful irregular erosions involving the most commonly the buccal and palatal region but the lips, the pharynx, the esophagus may also be involved. In patients who present with isolated oral lesions the diagnosis is often delayed. Other mucosae including the conjunctival, nasal, urethral, vulval and cervical can also be involved. Cervicovaginal erosions are present in 51% of women with pemphigus vulgaris.(75)

Cutaneous lesions may be localized but more often are generalized. In the mucocutaneous type the mucosal lesions may precede the cutaneous involvement by an average of 5months

duration. The cutaneous lesions are flaccid, thin-walled vesicles and bullae on otherwise normal appearing skin. The blisters being fragile rupture easily leaving oozing erosions that have a tendency to extend peripherally. The sites predominantly involved are flexural sites such as the axillae and the groin, the face and the scalp and sites prone to friction such as elbows. A characteristic sign can be elicited in pemphigus is the separation of normal-appearing epidermis from the dermis by a firm sliding pressure. This sign known eponymously as Nikolsky's sign is characteristic of active disease. The erosions heal with hyperpigmentation but without scarring. Nail involvement presents as acute paronychia or rarely subungual hematoma.(2,75)

Pemphigus vegetans is a variant of pemphigus vulgaris. Vegetating papillomatous plaques are seen especially in the intertriginous regions. Two subtypes have been described: Neuman type and the Hallopeau type. The Neuman type is more severe with the initial lesions being clear vesicles. The initial lesions in the mild Hallopeau type are pustules and the vegetative plaques may be studded with pustules.(2)

***Pemphigus foliaceus and its variants***

Pemphigus foliaceus presents with scaly crusted erosions often on erythematous base. The lesions are generally well demarcated in early disease. The primary lesions that is superficial flaccid vesicle is usually not seen. The mucosa is not involved. Patients with pemphigus foliaceus may have disease limited mainly to the scalp and the seborrheic areas or may occasionally progress to erythroderma.(76) Pemphigus foliaceus affects middle-aged individuals. The data regarding gender ratio in pemphigus foliaceus is not available because there are few studies that have looked at pemphigus foliaceus alone.

Pemphigus erythematosus is a variant of pemphigus foliaceus. The characteristic feature is the presence of erythematous scaly lesions over the nose and cheeks, in butterfly distribution of face. All patients with this variant have immunofluorescence pattern of pemphigus along with the presence of IgG and complement in the basement membrane zone similar to the lupus band. Approximately 30% have positive antinuclear antibodies. However the development of lupus erythematosus in these patients is extremely rare and the disease course is similar to pemphigus foliaceus.(1,76)

Pemphigus herpetiformis is an uncommon variant of pemphigus foliaceus. Itchy urticarial lesions with clusters of vesicles in herpetiform pattern are seen. The histopathology in this condition rarely demonstrates acantholysis. The immunofluorescence is usually similar to pemphigus foliaceus. Rarely the herpetiform pemphigus may evolve into pemphigus vulgaris.(1,2)

***Induced pemphigus***

Induced pemphigus commonly presents in the clinical pattern of pemphigus foliaceus or erythematosus. Pemphigus vulgaris is rarely seen as a manifestion of induced pemphigus. The ratio of induced foliaceus to induced vulgaris is approximately 4:1. Thiol –induced pemphigus resolves after withdrawal of the drug in 40-50% of patients, whereas among the non-thiol induced pemphigus only 15% cases remit after drug withdrawal.(1)

***Assessing the severity of pemphigus***

Various scoring systems have been devised that can be used to assess clinical severity of pemphigus. The Pemphigus Disease Area Index (PDAI) is one such scoring system. PDAI is

the result of a consensus of International Pemphigus Committee meetings in 2009 that were held to develop a tool to measure pemphigus disease activity. PDAI integrates cutaneous with mucosal disease in well-defined anatomical locations, assesses size and number of lesions and also scores the damage as seen by post inflammatory hyperpigmentation. Active lesions on skin and mucosae are each scored from 0 to 10 based on size and number of lesions and 12 different anatomical sites are identified and scored individually giving a maximum score of 120 each for skin and mucosa. The scalp region is scored separately with range of score from 0 to 10. Damage score is given as 1 point for presence of pigmentation for each of the 12 anatomical regions on the skin and 1 for the scalp thus giving a maximum score of 13. The total PDAI score can vary between 0 to 263.(77)

Other scoring systems include the Autoimmune Bullous Skin Disorder Intensity Score (ABSIS), and Pemphigus Vulgaris Activity Score (PVAS). ABSIS was developed by Pfutze et al and has been used widely in European studies. The score is calculated as sum total of skin scores, mucosal scores and objective score for oral intake as tolerated by the patient. The skin score can be a maximum of 150 points as given by assessing the extent and body surface area involvement which is weighted by the type of lesions. The mucosal involvement is given upto 11 points and the qualitative score given for oral discomfort can range from 0 to 45. The ABSIS score ranges from 0 to maximum of 206.(78) PVAS was introduced by Chams-Davatchi et al. The score ranges from 0 to 18 and includes a point system scoring for extent of mucocutaneous involvement, the type of lesions and Nikolsky's sign. The score is easy to use and was found to correlate well with physicians global assessment.(79) Indian scoring systems that have been developed include the Kumar's scoring system wherein skin and mucosa are graded separately as no disease, minimal disease, to severe disease giving a score of 0 to 3.(60) Saraswat et al presented a scoring system for oral pemphigus that took into

account extent of involvement as an objective score ranging from 0 to 11 and a subjective score for severity based on patient's discomfort. This score was similar to ABSIS with respect to inclusion of subjective discomfort of patient to oral diet, which was scored from 0 to 45.(78) In the Mahajan's scoring system for pemphigus, disease activity is graded from mild to extensive based on body surface area involvement of skin, extent of oral involvement and the individuals functional impairment.(80) Harman's grading of pemphigus ranges from 0 to 3 each for the skin and mucosa based on the number of erosions.(81)

Rosenbach et al, in their study done to validate the PDAI score, have concluded it to be more reproducible and to correlate better with physician's assessment of extent of disease in comparison to ABSIS score.(82) Ten dermatologists scored 15 pemphigus patients using both the PDAI and ABSIS scores. They also repeated the scoring assess reproducibility of the measurement tool. The inter-rater reliability was the same for both scores, but PDAI had better reproducibility as seen on analyzing the re-test. The PDAI scores correlated better with physician global assessment. In a recent study done by Rahbar Z et al, PDAI was compared with ABSIS as well as PVAS. The study was done on 100 patients, they were rated individually by 3 dermatologists for all three indexes PDAI, ABSIS and PVAS.(83) PDAI score was found to have better inter-rater reliability and better correlation with desmoglein tires of patients. Thus the PDAI has been consistently found to be a superior scoring system for the measurement of disease activity in pemphigus.

# MATERIALS AND METHOD

**Study design**

The study was a hospital based cross-sectional study of patients with pemphigus admitted in dermatology ward or attending the dermatology outpatient department.

**Setting**

The study was conducted in the Department of Dermatology, Venereology and Leprosy at Christian Medical College, a tertiary level hospital in Vellore, Tamil Nadu.

**Study duration**

The study was conducted between November 2013 and August 2014 (10 months).

**IRB approval**

The study was approved by the Institutional Review Board. (IRB approval no.8549)

**Inclusion criteria for cases :**

1. Patients with pemphigus vulgaris diagnosed by clinical features, histopathology and immunofluorescence or serological demonstration of anti-desmoglein antibodies and consenting for the study.
2. Patients with pemphigus foliaceus diagnosed by clinical features, histopathology and immunofluorescence or serological demonstration of anti-desmoglein antibodies with active disease were included for studying the secondary objective i.e. correlation between PDAI score and IgG4 levels.

**Exclusion criteria for cases :**

1.Patients not willing to participate in the study.

2.Patients with paraneoplastic pemphigus.

**Inclusion criteria for Controls:**

1. Healthy renal transplant donors who were willing to participate in the study.

**Exclusion criteria for controls:**

1.Presence of autoimmune disease or immunobullous disease.

2.Family history of immunobullous disease.

**Sample size calculation for prevalence of HLA DR and DQ types in pemphigus vulgaris**

The sample size was calculated in the following way to show a difference in HLA allele of about 30% across patients and controls 80% of the time with 5% level of significance.

The prevalence data used for the calculation of sample size was from the study by Delgado et al.(34)

$$n = \frac{(Z_{\alpha/2} + Z_{1-\beta})^2 \ X \ 2 \ X \ PQ}{d^2}$$

$P = (P_1 + P_2)/2$
$P = (48+17)/2 = 32.5$

$Q = 100-32.5 +67.5\%$
$D = 48-17 = 30\%$

$Z_{\alpha/2} = 1.96$ (5% level of significance)
$Z_{1-\beta} = 0.84$ (80% power)

Where, P – prevalence from the literature
P1(48) = prevalence in cases
P2(17)= prevalence in controls.
Q = 1-P
$Z_{\alpha/2}$ is the standard normalized value at 5% level of significance

The sample size calculated was **45 cases** and **45 controls**.

**Methodology**

Patients with established diagnosis of pemphigus vulgaris were enrolled in the study after informed consent was obtained by the principal investigator.

The demographic details collected included the age, sex, area of residence. History pertaining to the disease included the duration of disease, the anatomical site of onset of lesions, the symptoms associated at the onset of lesions, the presence of photosensitivity, the history of drug intake that may have triggered the disease. Duration of disease in this study was the time duration from onset of symptoms till the inclusion in study.

The details of treatment received by the patients was ascertained by scrutiny of previous records and when not available the history was relied upon and recorded. The total duration of steroid, maximum dose of steroid received by the patient and the current dose were recorded. The details of other steroid sparing immunosuppresants that the patient had been on, with the average duration and dosage of each of the them were also recorded.

History of associated comorbid conditions were recorded.

In patients with active disease the skin, scalp and mucosae was examined. The type of lesions on skin such as vesicles, bullae, erosions, vegetating plaques, acanthoma, post-inflammatory macules and their distribution were recorded. The extent of oral mucosal involvement and the involvement of other mucosa such as the conjunctiva, nasal and anogenital was also recorded. Nail involvement in the form of paronychia was noted. Nikolsky sign was performed in patients with active disease, as was bulla spread sign in patients with vesicular lesions.

The severity of disease was objectively assessed using **Pemphigus Disease Area Index** (PDAI) (Annexure 2). Total PDAI score was calculated by sum of scores given as per size of lesions and number of lesions present on the skin, mucosa and scalp. Activity score was given for presence of erosions or bullae. It was calculated as sum of scores for 12 anatomical areas. The score in each area was given as 0, 1, 2, 3, 5 or 10. The activity score for the scalp was given as 0, 1, 2, 3, 4 depending on the number of quadrants involved or as 10 if the entire scalp was involved. Damage score was given for each of the 12 skin areas and 1 for the scalp. Damage score of 1 was given for presence of hyperpigmentation or resolving erythema. Activity score for mucosal involvement was given as 0, 1, 2, 5 or 10 based on the number and size of erosions or blisters involving 12 sites. The oral mucosa was given maximum weightage and constituted 8 of the 12 sites and the other 4 mucosal sites included eyes, nose, posterior pharynx and anogenital. Activity score for skin can range from 0 to 120, scalp score can range from 0 to 10, damage score 0 to 13 and mucosal score ranged from 0 to 120. Thus the total PDAI can range from 0 to 263.

**Investigations**

Laboratory investigations included Tzanck smear from fresh erosion or early vesicle, skin biopsy of early vesicular lesion if present, direct immunofluorescence of perilesional skin. Serum anti-desmoglein1 and anti-desmoglein 3 antibody titres were done at the first visit by ELISA.

**HLA DR and DQ typing**

HLA DR and DQ typing was done in pemphigus vulgaris patients.

Sample: 10mL blood sample was collected in an citrated tube by venepuncture of peripheral vein and DNA extracted by salting-out technique.

The HLA DR and DQ typing was done by the Polymerase Chain Reaction Sequence Specific Oligonucleotide Probe method using Life Code SSO HLA typing kits with Luminex, product of Gen Probe Transplant Diagnostics Inc, Stamford, US.

Principle of PCR SSOP : The selected DNA region for the specific genes to be detected is enriched by the polymerase chain reaction. Primers are used to generate excess of single stranded DNA material. Probes that are oligonucleotides homologous to specific sequences on the amplified DNA, i.e. the alleles that need to be identified, are hybridized with the PCR product. These probes are attached to microspheres that can be identified by their specific fluorescence. The fluorescence of multiple probes can be read at the same time. The resultant measurement of is given as positivity or negativity for a particular probe for the amplified DNA sample.

**Serum total IgG4**

Serum total IgG4 levels were done in all patients with active disease at the time of recruitment in the study.

Sample- 3-5 mL blood sample was collected by venepuncture of peripheral vein. Blood was allowed to clot and the serum separated as soon as possible to prevent haemolysis. The sera was then stored at 2-8°C.

Serum IgG4 levels were measured by Nephelometry method using the MININEPH Human IgG4 kit, a product of The Binding Site Group Ltd, Birmingham, UK.

Principle of nephelometry : The amount of light that is scattered by a solution depends on the amount of particles that are suspended in it. The IgG4 are protein molecules that are soluble in the serum. Addition of antibodies to them makes them form small antigen-antibody complexes that remain in suspension . The amount of light that is scattered will be directly proportional to the concentration of the IgG4 in the sample. This amount of scattered light is then measured and compared with the light scattered from known solutions as given by a standard calibration curve.

The normal serum IgG4 levels in adults ranges from 62 to 1127 mg/L.

**Statistical analysis**

The data was entered in Epidata version 3.1, and analyzed using the software SPSS version 17. Continuous variables were analyzed and represented by using mean and standard deviations. Categorical variables like gender, type of pemphigus were described using frequencies with percentages.

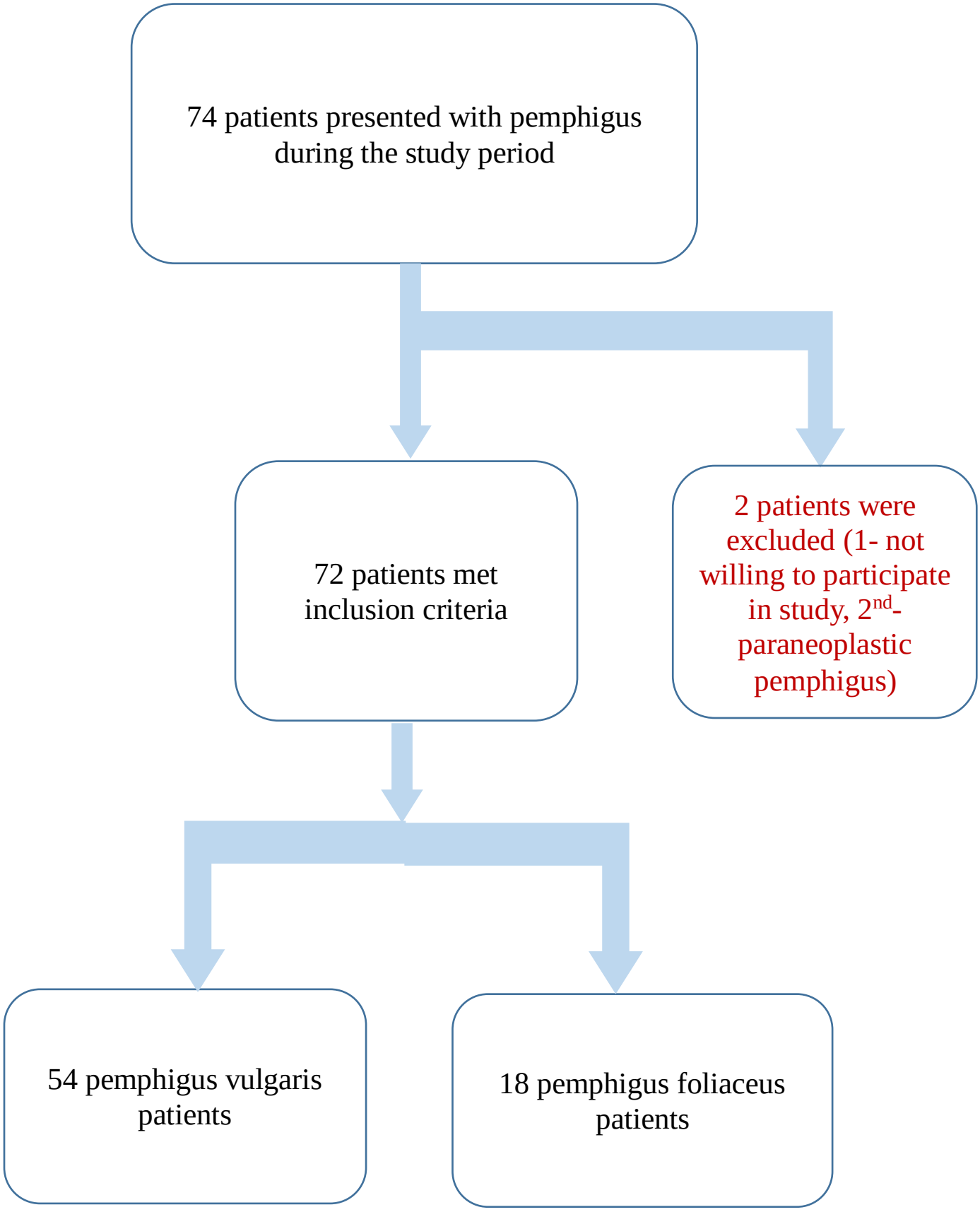
The HLA allele frequencies in cases and controls were compared using the Chi-square test. Spearman Rho correlation coefficient was used to correlate PDAI scores with serum total IgG4 levels, PDAI scores with anti-desmoglein titres and IgG4 with anti- desmoglein titres. It was diagrammatically represented on scatter plots.

The Mann-Whitney U test for used to analyse the relation of HLA DR and DQ types with disease severity.

# RESULTS

Seventy-seven patients with pemphigus were seen during the period of study from November 2013 to August 2014. The diagnosis was not confirmed in 3 clinically suspected pemphigus cases. This was due to failure to follow up for investigations. One patient was diagnosed to have paraneoplastic pemphigus therefore excluded from study. One patient was not willing to participate in the study. Seventy two patients consented to participate in the study. Of the 72 patients, 30 were newly diagnosed cases. The proportion of patients with pemphigus among new dermatology attendees during the study period was 0.5% (30 / 5796 patients).

Fifty-four (75%) patients had pemphigus vulgaris (75%) of whom 3 patients had pemphigus vegetans and 18 (25%) patients had pemphigus foliaceus.


74 patients presented with pemphigus during the study period
72 patients met inclusion criteria
2 patients were excluded (1- not willing to participate in study, 2nd- paraneoplastic pemphigus)
54 pemphigus vulgaris patients
18 pemphigus foliaceus patients

**Demographic profile of patients**

The mean age at presentation of patients with pemphigus was 43.19± 14.06 years (range, 12 - 70 years). There were 36 (50%) females and 36 (50%) males (M;F ratio 1:1). The frequency distribution of patients in different age groups with gender is given in ***figure 1.*** Thirty percent of the patients were in the age group of 41 -50 years.

The mean age at onset of disease in patients with pemphigus vulgaris was 39.25 ± 13.33 years (range, 12- 68 years). In 26 patients (48.1%) the onset of disease was before the age of 40 years and in 4 of them the age at onset was 20 years or less.

Among the pemphigus vulgaris patients there was slight female predominance, 32 females and 22 males (M:F ratio was 1:1.45).

In the patients with pemphigus foliaceus the age at onset was higher, mean age of 44.36 years ±12.9 (range, 24 - 66 years). However in the pemphigus foliaceus group of patients there was a high proportion of males compared to females with M:F ratio of 3.5:1.

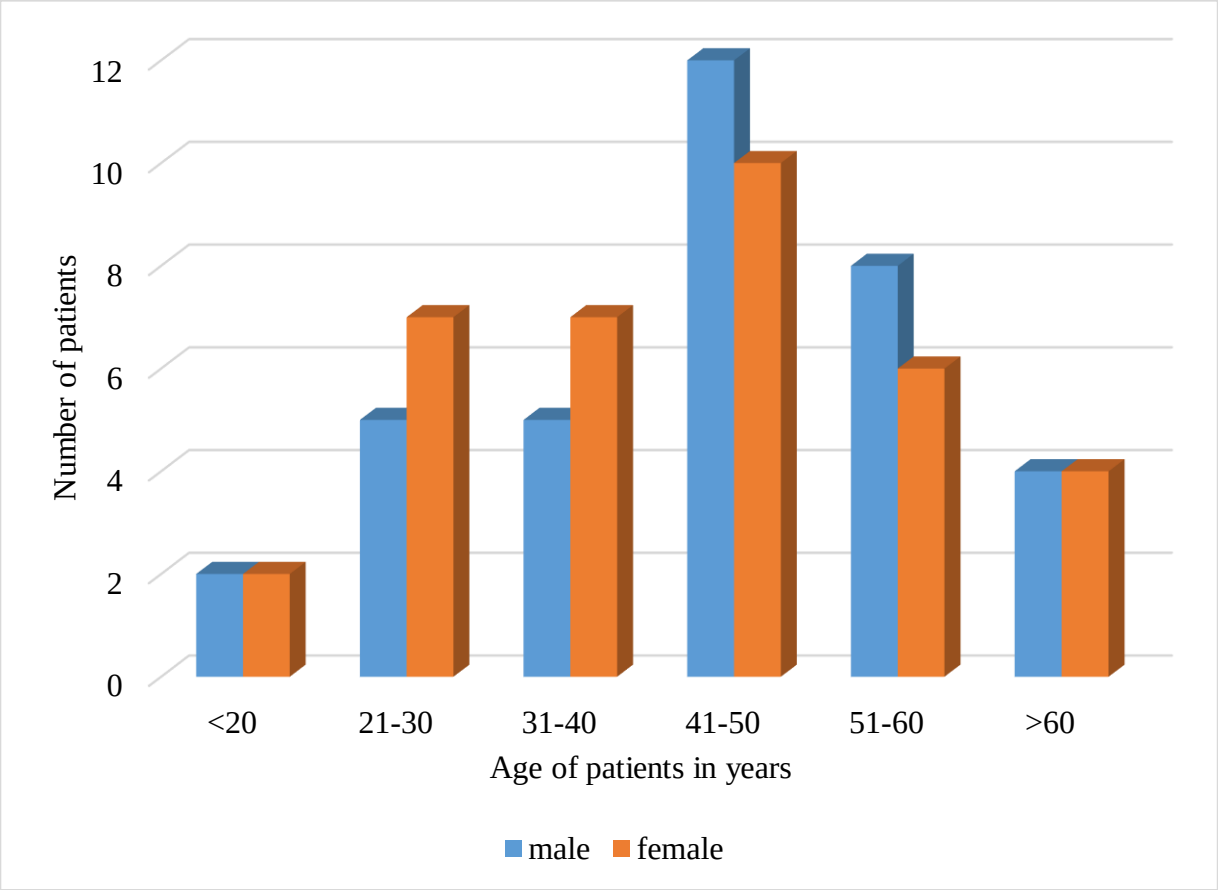
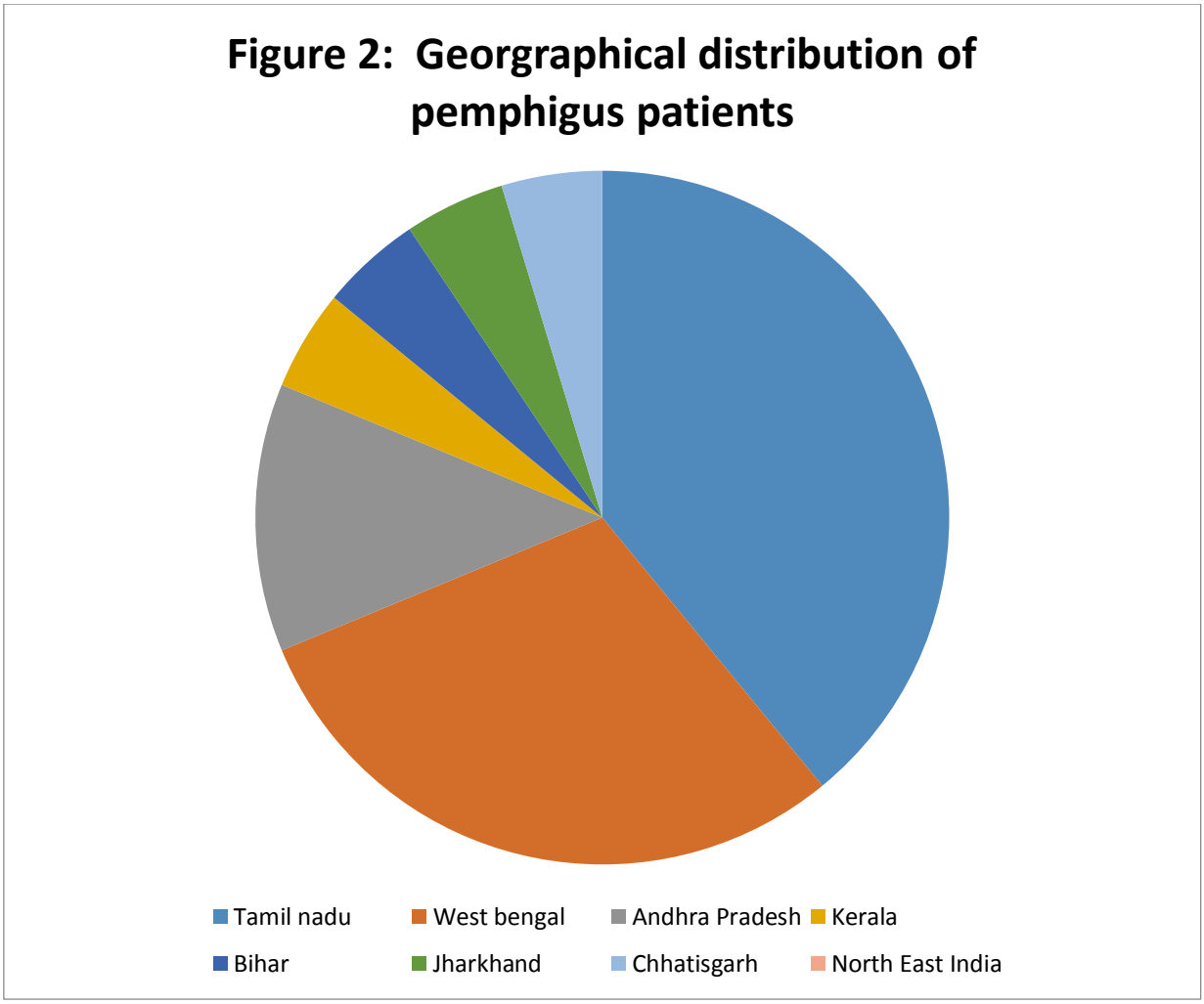



**Figure 1: Age and sex distribution of pemphigus patients**

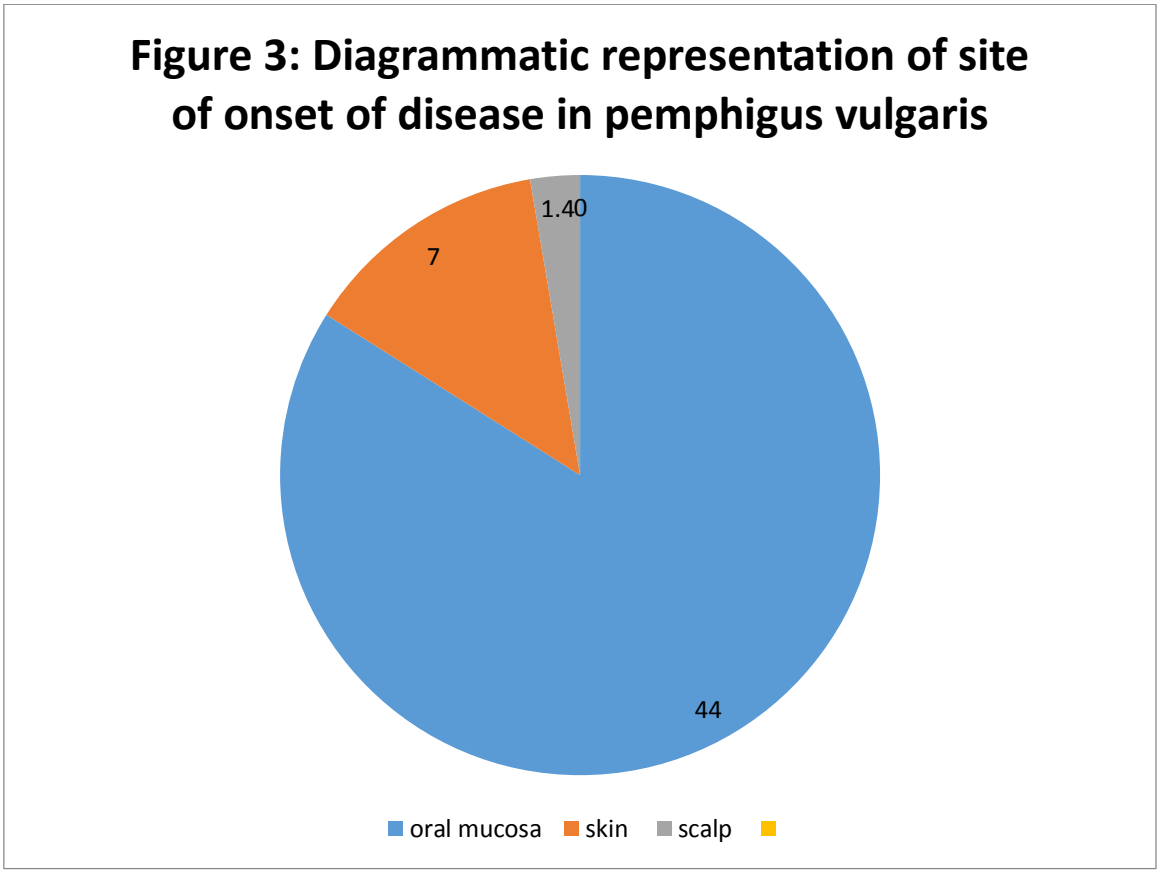



Majority of the patients were from Tamil Nadu (n=25), followed in frequency by West Bengal (n=19), Andhra Pradesh (n=8) and 3 patients each from Kerala, Bihar, Jharkhand, Chhatisgarh and the remaining 8 patients were from different states of the North-East India. The geographical distribution of patients is as represented in ***figure 2.***

**Clinical profile of patients :**

**Site of involvement :**

The oral mucosa was the first site of onset of disease in 44 (81.6%) of the pemphigus vulgaris patients, the skin and scalp were the first site to be involved in 5 (9.2%) patients each as represented in ***figure 3.*** Among the pemphigus foliaceus patients skin was the first site of onset of disease in 12/18 (66.7%), in the remaining 6/18 (33.3%) patients the disease first began on the scalp.



**Duration of disease prior to inclusion into the study**

The duration of disease prior to inclusion in the study ranged from 2 months to 15 years with median duration of 2.5 years. The duration of disease in pemphigus vulgaris patients was 2.9 ± 2.7 years and in pemphigus foliaceus was 2.4 ± 1.9 years.

**Associated symptoms**

Pain was the most common symptom at presentation at disease onset, among patients with pemphigus vulgaris (48.1%) whereas pruritus was the most common symptom in patients with pemphigus foliaceus (57.9%). The frequency of presenting symptom in pemphigus vulgaris and foliaceus patients is given in ***table 4.***

**Table 4-Associated clinical symptoms in patients with pemphigus vulgaris and pemphigus foliaceus**

| **Symptom** | **Number of patients (percentage)** | |
|---|---|---|
| | **Pemphigus vulgaris (n=54)** | **Pemphigus foliaceus (n=18)** |
| Pain | 26 (48.1%) | 2 (11.1%) |
| Burning | 8 (14.8%) | 1 (5.5%) |
| Itching | 5 (9.3%) | 11 (61.1%) |
| Pain and burning | 5 (9.3%) | 0 |
| Pain and itching | 7 (12.9%) | 1 (5.5%) |
| No symptom | 3 (5.6%) | 3 (16.6%) |

History of photosensitivity was elicited in 6 out of the 54 pemphigus vulgaris patients and 3 of the 18 pemphigus foliaceus patients.

**Drug inducing pemphigus**

The history of drugs triggering pemphigus was obtained in one patient of pemphigus vulgaris and the probable drug implicated was angiotensin convertase inhibitor enalapril.

**Prior treatment of pemphigus**

Majority of the pemphigus patients (n=68, 94.4%) in our study had been on treatment with specific therapy prior to their inclusion in this study and all of them were being treated with steroid with or without other immunosuppresants. The steroid-sparing immunosuppressants used included azathioprine, cyclophosphamide, mycophenolate mofetil, methotrexate and dapsone. One patient of pemphigus vulgaris had received IVIG in the past. Azathioprine was the most frequently (50.7%) used steroid sparing agent.

The frequency distribution of the immunosuppressants used and their average duration of use is given in ***table 5.***

Of the 68 patients who were on steroid therapy 54 had received only oral steroid, 12 had received pulse steroid with cyclophosphamide in the past, 1 patient each received oral and intravenous steroids and 1 patient had received oral and intramuscular steroids. The maximum dose of oral steroid used to treat patients ranged from 0.2 – 1.75mg/kg, with average dose of 0.9mg/kg (± 0.36mg/kg).

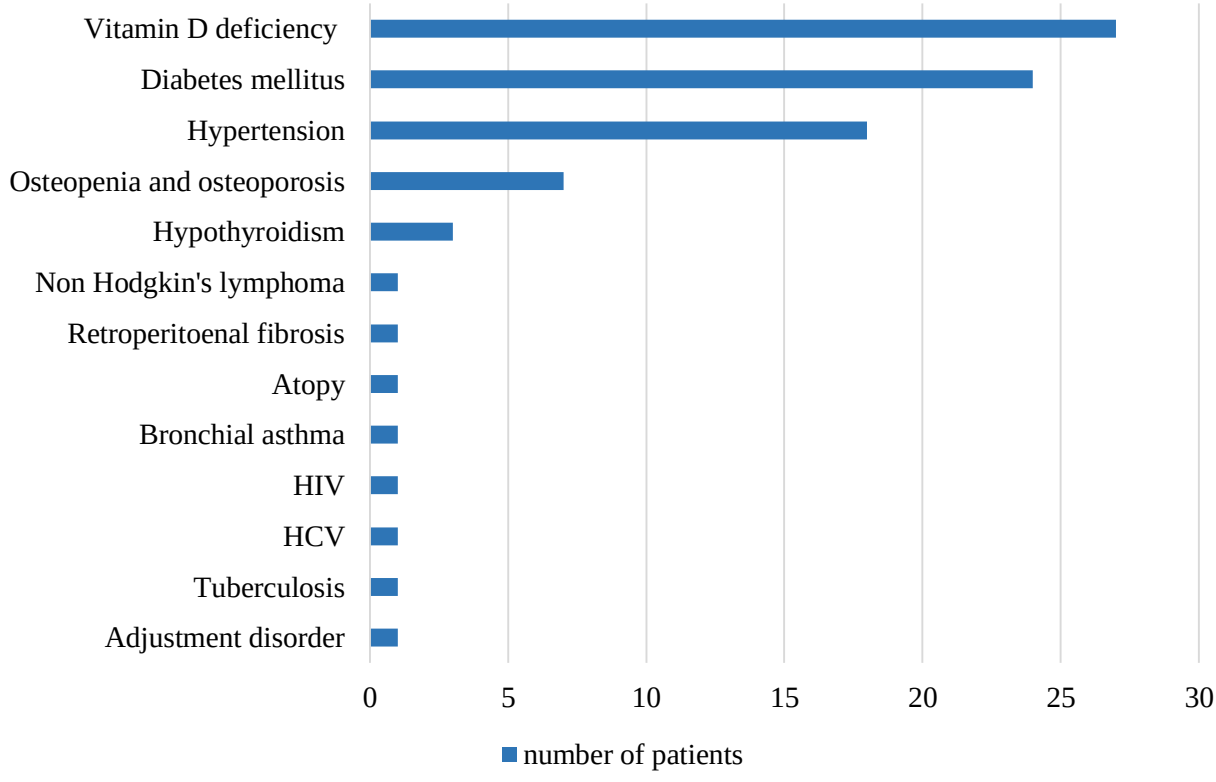
**Table 5- Steroid-sparing immunosuppressants used in patients prior to inclusion in study**

| Immunosuppresant /adjuvant used | Number of patients (percentage) | Average duration of treatment in years (range) |
|---|---|---|
| Azathioprine | 37 (50.6%) | 1.22 (0.03-5 ) |
| Cyclophosphamide | 13 (17.8%) | 0.85 (0.02 -5) |
| Mycophenolate mofetil | 2 (2.7%) | 0.41 (0.08-0.75) |
| Methotrexate | 5 (6.8%) | 0.84 (0.08-2) |
| Dapsone | 11 (15.0%) | 0.82 (0.08- 4) |

**Associated comorbid conditions**

Fifty two of the 72 patients (72.2 %) had other associated comorbidities of which vitamin D deficiency was the most common (n=27, 35.5%), followed in frequency by diabetes mellitus seen in (n=24, 33.3%). Hypertension was present in 18 patients (25%), hypothyroidism was seen in 3 patients (4.2%), osteoporosis and osteopenia was seen in 7 patients (9.7%). HIV and HCV was positive in one patient each, and bronchial asthma, atopy, adjustment disorder, and past history of tuberculosis were seen in one patient each. ***(Figure 4)*** A single patient had retroperitoneal fibrosis and presented with clinical and DIF features of pemphigus vulgaris. Subsequently, six months later she was diagnosed to have non-Hodgkin's lymphoma.

**Figure 4: Bar chart representation of the frequency of comorbid conditions in patients with pemphigus**



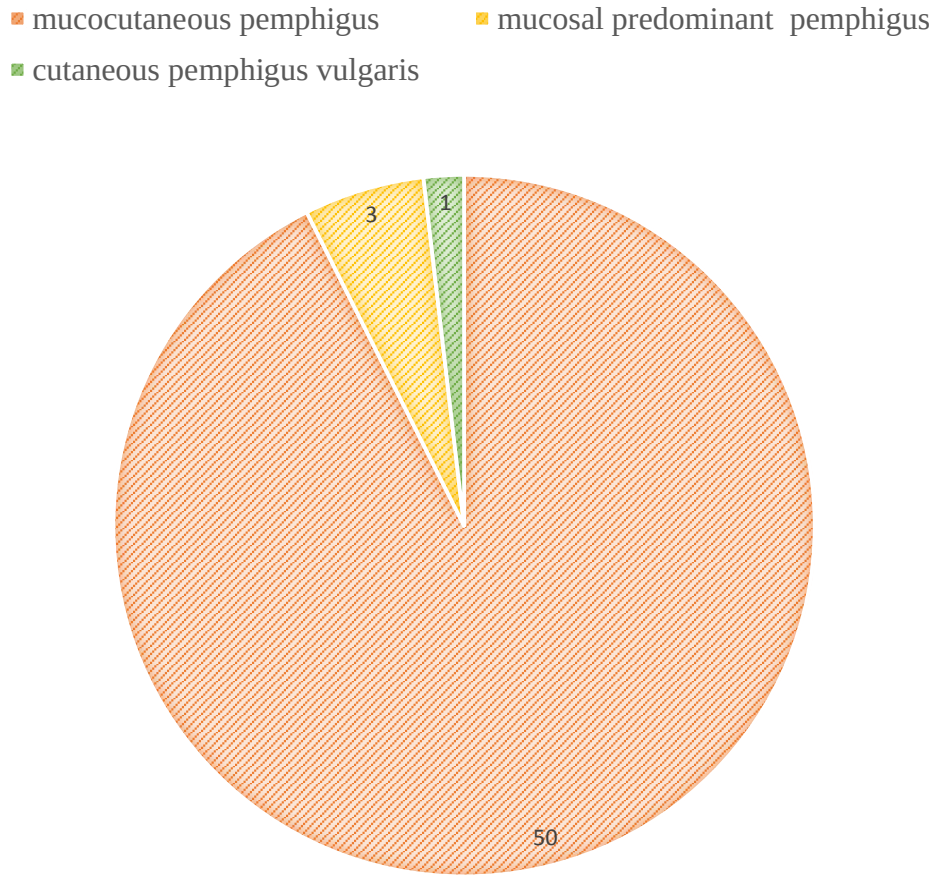
**Family history of pemphigus**

Family history of pemphigus was not present in any patient.

**Clinical profile**

Of the 54 patients of pemphigus vulgaris, disease was active in 42 patients and 10 patients were in remission. In patients with active disease, the oral mucosa was the most common site of involvement in patients with pemphigus vulgaris (35/42, 83.3%) Other mucosal involvement was present in 7 patients (16.6%), 5 patients had genital mucosal involvement (11.9%), 1 patient each had eye and nasal mucosal involvement. Flexural involvement was seen in 10 patients (23.8%). Paronychial involvement was seen in 7 patients (16.6%). Nikolsky sign was positive in 6 of the 33 (18.8%) pemphigus vulgaris patients in whom skin lesions were present. Majority of patients had mucocutaneous type of pemphigus (92.56%), 3 patients had mucosal pemphigus (5.56%) and 1 patient had cutaneous type of pemphigus vulgaris (1.8%). ***(Figure 5)***

**Figure 5: Clinical types of pemphigus vulgaris**



Of the 18 pemphigus foliaceus patients skin lesions were present in all the patients and scalp was involved in 13 cases and one patient had paronychial involvement. One patient of pemphigus foliaceus was in erythroderma. Nikolsky sign was positive in 9 patients (50%). The frequency of distribution of skin lesions in both the groups is detailed in ***table 6.***

**Table 6- Distribution of lesions in pemphigus patients in this study**

| Site involved | Number of pemphigus vulgaris patients (percentage) | Number of pemphigus foliaceus patients (percentage) |
|---|---|---|
| **Skin** | | |
| Face | 20 (47.6) | 13 (72.2) |
| Upper limbs | 21 (50.0) | 17 (94.4) |
| Lower limbs | 16 (38.0) | 9 (50.0) |
| Trunk | 31 (73.8) | 17 (94.4) |
| Fexures | 11 (26.1) | 6 (33.3) |
| **Mucosa** | | - |
| Oral | 35 (83.3) | |
| Eye | 1 (2.3) | - |
| Nasal | 1 (2.3) | - |
| Genital | 5 (11.9) | - |
| **Scalp** | 20 (47.6) | 13 (72.2) |
| **Nails** | 7 (16.6) | 1 (5.5) |

**PDAI score**

The mean PDAI score in pemphigus vulgaris patients was 24.1 with the minimum score of 1 and maximum score of 75. In the pemphigus foliaceus patients the PDAI score ranged from minimum of 3 to maximum of 105 with an mean score of 34.73.

**Table 7-The mean PDAI score of pemphigus vulgaris and foliaceus patients**

| **Parameter** | **Mean score (SD) in pemphigus vulgaris patients (n=42)** | **Mean score(SD) in pemphigus foliaceus patients (n=18)** |
|---|---|---|
| Activity score of skin (range, 0-120) | 9.83 (±14.01) | 26.17 (±29.48) |
| Damage score (range, 0-13) | 3.28 (±2.97) | 4.11 (± 3.14) |
| Activity score scalp (range, 0- 10) | 1.57 (±2.17) | 3.61 (±3.98) |
| Mucosal score (range, 0-120) | 8.88 (±13.03) | Not involved |
| Total PDAI score ( range, 0- 263) | 23.57 (±23.20) | 33.83 (±30.24) |

**Investigations**

Tzanck smear was positive for acantholytic cells in 19 patients in pemphigus vulgaris and 10 patients of pemphigus foliaceus. It was negative in 6 patients of pemphigus vulgaris and 4 patients of pemphigus foliaceus.

Antinuclear antibodies (ANA) was done in 3 out of 6 of the pemphigus vulgaris patients and 2 out of 3 of the pemphigus foliaceus patients who had history of photosensitivity. ANA was negative in all of 5 cases.

Biopsy was done at the time of diagnosis in 43/ 54 pemphigus vulgaris patients, of which 38 cases (88.4%) showed typical suprabasal bulla with acantholytic cells. In 5 patients the histopathology was not characteristic of pemphigus vulgaris. In these 4/5 cases the diagnosis was established by positive direct immunofluorescence and acantholytic cells on Tzanck smear and in the remaining case with high titres of anti-desmoglein antibodies. In the remaining cases where biopsy was not done, the diagnosis was either confirmed elsewhere prior to the patients visit to our hospital or by direct immunofluorescence and ELISA anti-desmoglein antibodies. Direct immunofluorescence of perilesional skin was done in 47/54 patients (87%). Of these 47, DIF was positive in 43/47 (91.4%) cases, in 42 cases (89.3%) intraepidermal immunoglobulin G was present, in 33 cases (70.2%) complement (C3) was also present. In one case IgG was negative but C3 was present. The most common pattern of IgG was intercellular deposit of lower one third of epidermis and IgG was negative in 5 cases. IgG was deposited along basement membrane in one case of pemphigus vulgaris, but the clinical features were suggestive of pemphigus vulgaris and there was no evidence of

associated malignancy. Complement (C3) deposit was more often located in the lower third of the epidermis. Complement deposit was not seen in 14 cases. (***Table 8)***

In the pemphigus foliaceus group of patients, biopsy was contributory to diagnosis in all 12 patients in whom biopsy was done. Direct immunofluorescence was positive for intraepidermal IgG in all the 18 pemphigus foliaceus patients. IgG deposits were present in entire thickness of the epidermis in 12 cases (66.6%) and in the lower half of the epidermis in 6 cases (44.4%). Complement deposit was present in lower third of the epidermis in 8 patients (44.4 %) and the entire thickness of epidermis in 7 cases (38.8%). ***(Table 9)***

A summary of the direct immunofluorescence findings in our study in given in ***table 10.***

**Table 8-Diagnostic investigations done in patients with pemphigus vulgaris**

| Study number | Tzanck smear | Biopsy | IgG deposit on DIF | Complement deposit on DIF | Serum Anti-Dsg1 in u/mL (normal 2-20u/mL) | Serum Anti-Dsg3 in u/mL (normal 2-20u/mL) |
|---|---|---|---|---|---|---|
| 1 | Acantholytic cells | Pemphigus vulgaris | Present | Present | 210 | 156 |
| 2 | Negative | Pemphigus vulgaris | Present | Present | 147 | 173 |
| 3 | Negative | Not done | Not done | Not done | 135 | 161 |
| 4 | Not done | Pemphigus vulgaris | Present | Absent | 85 | 155 |
| 5 | Negative | Pemphigus vulgaris | Present | Present | 2 | 122 |
| 6 | Acantholytic cells | Pemphigus vulgaris | Present | Present | 13 | 160 |
| 7 | Not done | Pemphigus vulgaris | Present | Present | 6.3 | 7.1 |
| 8 | Not done | Not characteristic | Not done | Not done | 49 | 143 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 9 | Not done | Not done | Not done | Not done | 7.6 | 170 |
| 10 | Not done | Not done | Not done | Not done | 5.5 | 211 |
| 11 | Not done | Not characteristic | Present | Absent | 43 | 202 |
| 12 | Not done | Pemphigus vulgaris | Present | Present | 12.2 | 176 |
| 13 | Acantholytic cells | Pemphigus vulgaris | Present | Absent | 2 | 2 |
| 14 | Not done | Not done | Not done | Not done | 15.2 | 122 |
| 15 | Not done | Pemphigus vulgaris | Present | Present | 1.5 | 99 |
| 16 | Acantholytic cells | Not done | Absent | Absent | 8 | 166 |
| 17 | Not done | Pemphigus vulgaris | Present | Absent | 182 | 185 |
| 18 | Not done | Pemphigus vulgaris | Present | Absent | 2 | 67.4 |
| 19 | Not done | Pemphigus vulgaris | Present | Present | 2 | 143 |
| 20 | Not done | Not done | Present | Absent | 4.5 | 187 |
| 21 | Not done | Pemphigus vulgaris | Present | Present | 190 | 202 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 22 | Not done | Pemphigus vulgaris | Present | Present | 15 | 4.2 |
| 23 | Not done | Not done | Present | Present | Not done | Not done |
| 24 | Acantholytic cells | Pemphigus vulgaris | Absent | Absent | 3.8 | 4.8 |
| 25 | Acantholytic cells | Pemphigus vulgaris | Present | Present | 148 | 169 |
| 26 | Acantholytic cells | Not characteristic | Present | Present | 2.3 | 195 |
| 27 | Acantholytic cells | Not characteristic | Present | Absent | 2 | 16.4 |
| 28 | Negative | Pemphigus vulgaris | Present | Present | 72 | 144 |
| 29 | Acantholytic cells | Pemphigus vulgaris | Present | Present | 2 | 14 |
| 30 | Acantholytic cells | Pemphigus vulgaris | Present | Present | 31.3 | 194 |
| 31 | Acantholytic cells | Pemphigus vulgaris | Present | Present | 256 | 200 |
| 32 | Not done | Pemphigus vulgaris | Present | Present | Not done | Not done |

| | | | | | | |
|---|---|---|---|---|---|---|
| 33 | Negative | Pemphigus vulgaris | Present | Absent | 5.6 | 79 |
| 34 | Negative | Pemphigus vulgaris | Present | Present | 161 | 144 |
| 35 | Acantholytic cells | Pemphigus vulgaris | Present | Absent | 29 | 198 |
| 36 | Not done | Pemphigus vulgaris | Absent | Present | 2 | 66 |
| 37 | Not done | Pemphigus vulgaris | Present | Present | 260 | 252 |
| 38 | Not done | Not done | Present | Present | 2.2 | 249 |
| 39 | Not done | Pemphigus vulgaris | Present | Present | 2 | 2 |
| 40 | Acantholytic cells | Pemphigus vulgaris | Present | Present | 2 | 11 |
| 41 | Not done | Pemphigus vulgaris | Absent | Absent | 68.5 | 76.3 |
| 42 | Acantholytic cells | Pemphigus vulgaris | Present | Present | 14.7 | 243 |
| 43 | Not done | Pemphigus vulgaris | Negative | Absent | 151 | 121 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 44 | Not done | Pemphigus vulgaris | Present | Present | 3.06 | 248 |
| 45 | Acantholytic cells | Pemphigus vulgaris | Present | Present | 9.2 | 257 |
| 46 | Acantholytic cells | Pemphigus vulgaris | Present | Present | 180 | 256 |
| 47 | Acantholytic cells | Not characteristic | Present | Absent | 66 | 250 |
| 48 | Negative | Not done | Present | Present | 127 | 261 |
| 49 | Negative | Pemphigus vulgaris | Present | Present | 37 | 221 |
| 50 | Not done | Not done | Not done | Not done | 51 | 249 |
| 51 | Acantholytic cells | Pemphigus vulgaris | Present | Present | 267 | 250 |
| 52 | Acantholytic cells | Pemphigus vulgaris | Present | Present | NA | NA |
| 53 | Not done | Pemphigus vulgaris | Not done | Not done | 2 | 19.4 |
| 54 | Not done | Not done | Present | Present | 5.3 | 94 |

*In patients with long standing disease where the diagnosis was established previously and the patient were on treatment Tzanck, biopsy and/or DIF were not repeated in our study. The anti-desmoglein levels contributed to the diagnosis and served as a measure for disease activity assessment in these cases.

**Table 9- Diagnostic investigations done in patients with pemphigus foliaceus**

| Study no | Tzanck test | Biopsy | IgG deposit on DIF | Complement deposit on DIF | Serum Anti-Dsg1 (Normal= 2-20u/mL) | Serum Anti-Dsg3 (Normal= 2-20u/mL) |
|---|---|---|---|---|---|---|
| 1 | Acantholytic cells | Pemphigus foliaceus | Present | Present | 146 | 2 |
| 2 | Acantholytic cells | Pemphigus foliaceus | Present | Absent | 200 | 2 |
| 3 | Acantholytic cells | Not done | Present | Present | 166 | 86 |
| 4 | Negative | Not done | Present | Absent | 163 | 2.1 |
| 5 | Acantholytic Cells | Not done | Present | Present | 234 | 117 |
| 6 | Negative | Pemphigus foliaceus | Present | Present | 26.4 | 2 |
| 7 | Negative | Notdone | Present | Present | 223 | 163 |
| 8 | Negative | Pemphigus foliaceus | Present | Present | 291 | 2.2 |
| 9 | Not done | Pemphigus foliaceus | Present | Present | 130 | 2.5 |
| 10 | Acantholytic cells | Pemphigus foliaceus | Present | Present | 204 | 32 |
| 11 | Acantholytic cells | Pemphigus foliaceus | Present | Present | 150 | 29 |
| 12 | Acantholytic cells | Not done | Present | Present | 234 | 94 |
| 12 | Acantholytic cells | Pemphigus foliaceus | Present | Present | 242 | 2 |
| 14 | Not done | Pemphigus foliaceus | Present | Absent | 136 | 2 |
| 15 | Acantholytic cells | Not done | Present | Present | 231 | 0 |
| 16 | Negative | Pemphigus foliaceus | Present | Present | 1 | 1 |
| 17 | Acantholytic cells | Pemphigus foliaceus | Present | Present | 234 | 33 |
| 18 | Not done | Pemphigus foliaceus | Present | Present | Not available | Not available |

**Table 10-Summary of Direct Immunofluorescence findings in pemphigus patients**

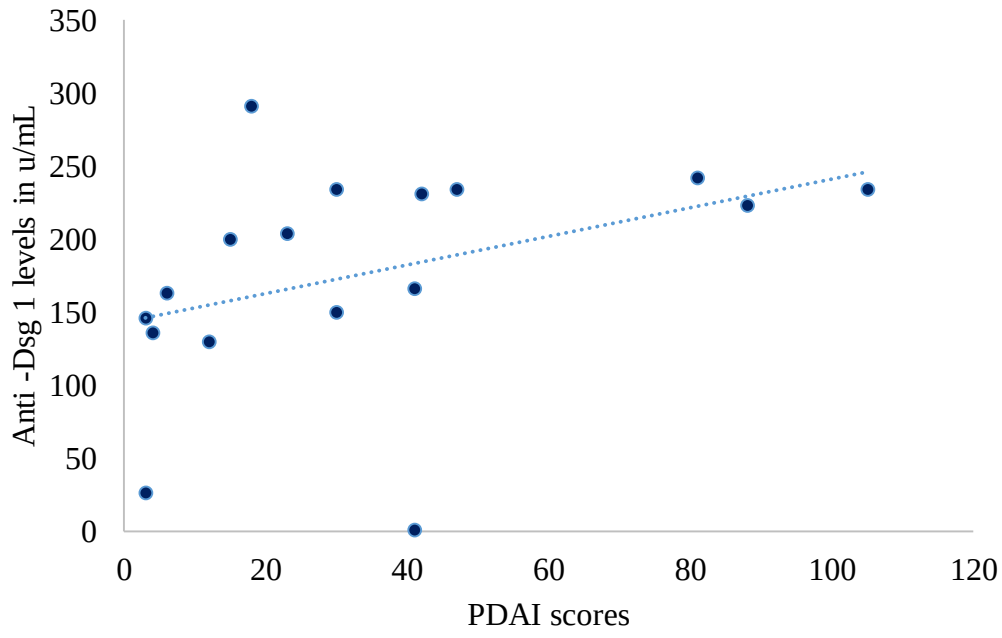
| Immunodeposit on DIF | Pemphigus vulgaris, n=47 (%) | Pemphigus folicaeus, n=18 (%) |
|---|---|---|
| IgG | | |
| Lower one third | 25 (53.2)) | 0 |
| Lower half of epidermis | 12 (25.5) | 6 (33.3) |
| Entire epidermal thickness | 4 (8.5) | 12 (66.6) |
| Basement membrane | 1 (2.1) | 0 |
| Total positive | 42 (89.3) | 18 (100) |
| Negative | 4 (14.8) | 0 |
| Complement C3 | | |
| Lower epidermis | 20 (42.6) | 8 (44.4) |
| Entire epidermal thickness | 0 | 7 (38.8) |
| Basal cells | 13 (27.6) | 0 |
| Total positive | 33 (70.2) | 15 (83.3) |
| Negative | 14 (29.7) | 3 (16.6) |

In the pemphigus vulgaris patients, serum desmoglein antibody levels were available for 52 patients, the mean (SD) anti-Dsg3 antibody titres was 147.01 (±82.80) u/mL. The anti-Dsg3 titres ranged from 2u/mL in patients in remission to maximum of 257 u/mL in patient with active disease. The mean anti–desmoglein1 antibody level was 60.8 u/ mL.

The mean (SD) anti-desmoglein 1 antibody level in pemphigus foliaceus patients was 177.14 (±78.22) u/mL. Anti-desmoglein 1 antibody level ranged from 1 to 291 u/mL.

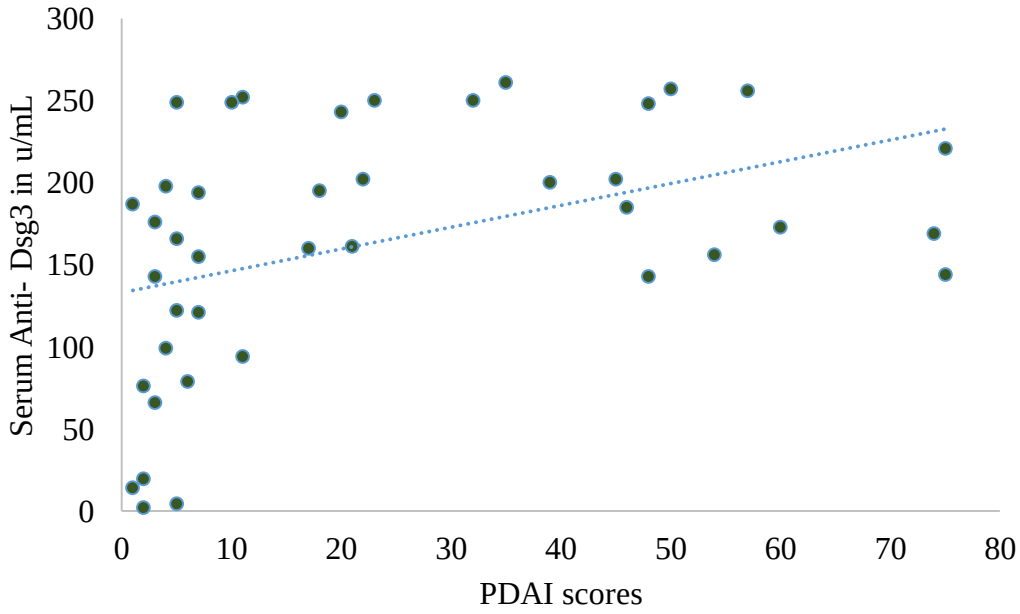
Correlation between anti-desmoglein 1 and PDAI in foliaceus patients showed a significant positive correlation. (R=0.54, p=0.02) as represented in the scatter plot in ***figure 6.***

**Figure 6: Correlation of anti-Dsg 1 levels with PDAI scores in pemphigus foliaceus**



Correlation between anti- desmoglein 3 and PDAI in pemphigus vulgaris patients showed a strongly significant positive correlation. (R=0.52, p=0.0004) as seen in scattered plot below in ***figure 7.***

**Figure 7: Correlation of anti-Dsg 3 with PDAI in pemphigus vulgaris**



**HLA types in the pemphigus vulgaris patients**

The required sample size (45) was achieved, we enrolled 50 cases of pemphigus vulgaris and 50 controls. Controls were taken from renal transplant donors. The mean age was 36 years (range, 19-57 years). There were 36 males and 14 females.

Forty-seven out of the 50 cases (94%) of pemphigus vulgaris had at least one HLA DRB1*14 allele, and 9 cases (18%) were homozygous for the allele. Among the controls HLA DRB1 *14 was present as at least one of the alleles in 18 controls (36%) and one was homozygous for DRB1*14. When compared with controls the difference in frequency of HLA DRB1*14 was found to be significant ($p < 0.001$). On the other hand HLA DRB1*15 was present in 15 of the controls and 5 controls were homozygous for DRB1*15, and it was present in 7/50 cases. The difference in frequency of DRB1*15 was significantly high in controls ($p = 0.04$). Nine controls were positive for DRB1*11, while only 2/50 patients were positive for it, resulting in significantly higher frequency of DRB1*11 in controls ($p < 0.05$). The other DRB1 alleles that we found in our pemphigus patients were DRB1*03, DRB1*04, DRB1*07, DRB1*08, DRB1*10, DRB1*12, DRB1*13, and DRB1*16. The frequency of the different HLA DRB1 alleles found in this study are given in ***table 11.***

Of the HLA DQB alleles, DQB1 *05 was seen in 47/50 patients (94%) and in 24/50 controls (48%) ($p < 0.001$). Ten cases (10/47) were homozygous for HLA DQB1*05. HLA DQB1*06 was found to be more common in the control population (18/50) than the patient group (8/50 cases) ($p = 0.02$). The other HLA DQB types that were seen in our patients with pemphigus were DQB1*02, DQB1*03, and DQB1*04. The frequency of HLA DQB1 alleles in patient and control group is given in ***table 12.***

Haplotype analysis revealed a high frequency of the haplotype HLA DRB1*14,DQB1*05 among the pemphigus patients (44 of 50, 88%), of these 7 patients were homozygous. Among controls the haplotype DRB1*14, DQB1*05 was seen in 14/50. This difference in frequency of this haplotype in cases versus controls was significant with $p < 0.001$. The haplotype DRB1*04, DQB1*03 was present in 12/50 cases (24%), none being homozygous for it. Among the controls 6/50 had the DRB1*04, DQB1*03 haplotype. The difference in frequency between the two groups was not statistically significant. We also found that haplotype DRB1*15, DQB1*06 was significantly more common in the control population. ***(Table 13)***

**Table11- HLA DRB1 alleles in pemphigus vulgaris patients as compared to healthy controls**

| DRB1 alleles | Pemphigus vulgaris(n=50) | Controls ( n=50) | P value |
|---|---|---|---|
| *01 | 0 | 3 | 0.08 |
| *03 | 1 | 2 | 0.56 |
| *04 | 13 | 8 | 0.24 |
| *07 | 8 | 14 | 0.17 |
| *08 | 4 | 1 | 0.17 |
| *09 | 0 | 1 | 0.31 |
| *10 | 2 | 5 | 0.24 |
| *11 | 2 | 9 | **0.02** |
| *12 | 3 | 10 | 0.045 |
| *13 | 2 | 6 | 0.149 |
| *14 | 47 | 18 | <0.001 |
| *15 | 7 | 16 | **0.03** |
| *16 | 1 | 0 | 0.316 |

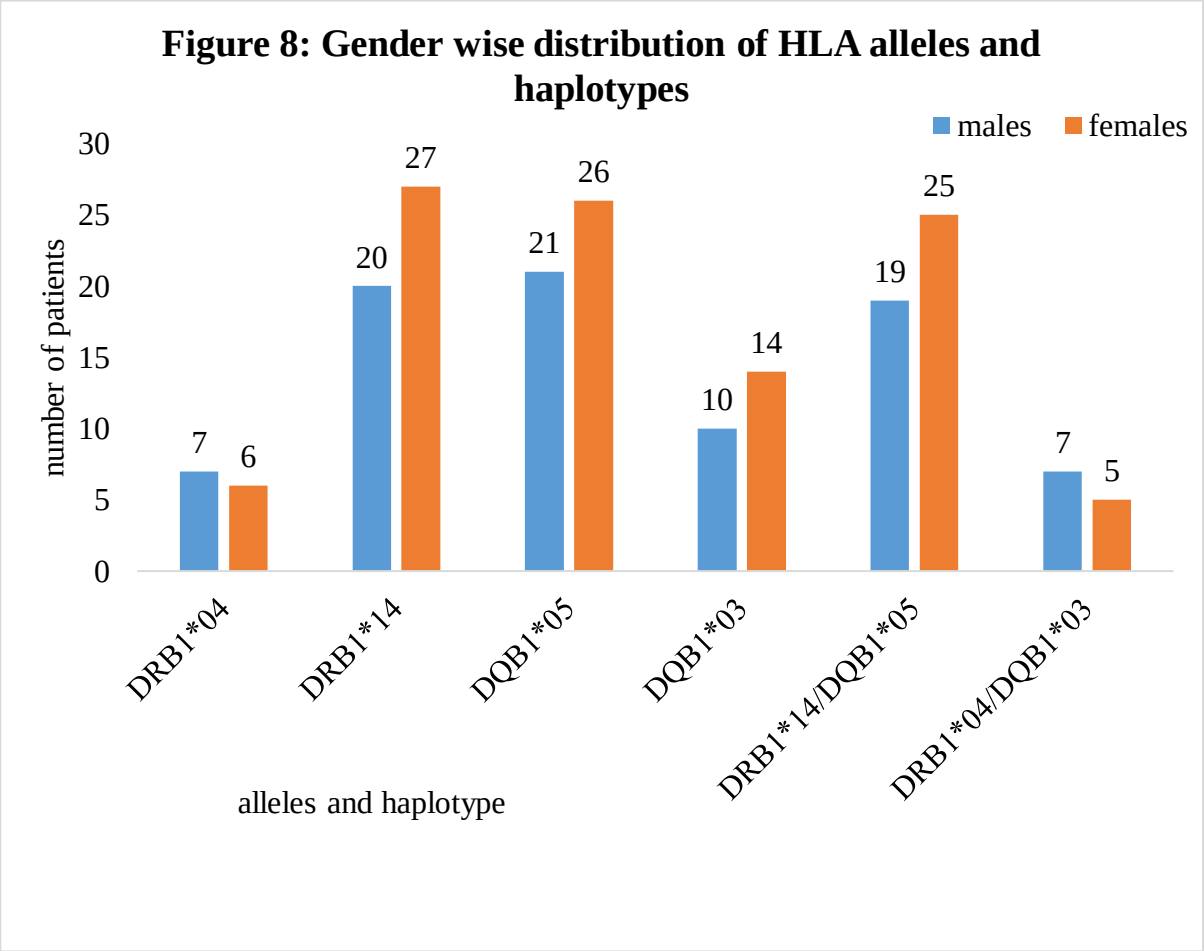
**Table 12- HLA DQB1 allele frequency distribution in pemphigus patients versus controls**

| DQB1 alleles | Pemphigus vulgaris (n=50) | Controls (n=50) | P value |
|---|---|---|---|
| *02 | 4 | 11 | 0.05 |
| *03 | 24 | 25 | 0.55 |
| *04 | 3 | 1 | 0.31 |
| *05 | 47 | 24 | <0.001 |
| *06 | 8 | 19 | **0.02** |

**Table 13- Comparison of haplotypes between pemphigus patients and healthy controls**

| Haplotype | Pemphigus patients | Controls | P value |
|---|---|---|---|
| DRB1*14/DBB1*05 | 44 | 14 | <0.001 |
| DRB1*04/DQB1*03 | 12 | 6 | 0.138 |
| DRB1*15/DQB1*06 | 5 | 15 | **0.018** |

As represented in the bar chart ***(figure 8)*** the allele types and haplotypes were distributed evenly in males and females. (There was no statistically significant difference based on gender).



**CORRELATION OF HLA TYPES WITH DISEASE SEVERITY**

The presence of HLA DQB1*03 (in 17/40 cases) was associated with significantly higher PDAI scores (mean score = 33.8) as compared to that of patients with absence of the allele (23/40 cases, mean PDAI = 16.6) with p value of 0.025.

The disease severity could not be compared in those with the presence of the allele and those in whom it was absent in case of HLA DRB1*14 and DQB1*05 because the alleles were present in almost all patients (39/40).

***Table 14*** summarizes the results of correlation between HLA DR, DQ alleles and haplotypes with severity of disease in pemphigus vulgaris patients.

**Table 14- The correlation between HLA DR and DQ alleles, haplotypes with disease severity**

| Allele /haplotype | Patients positive for the allele | | Patients negative for the allele | | P value |
|---|---|---|---|---|---|
| | No. of patients | PDAI score (mean ± SD) | No. of patients | PDAI score (mean ± SD) | |
| DRB1*14 | 39 | 24.4 ± 23.5 | 1 | 3 | 0.30 |
| DRB1*04 | 10 | 32.0 ± 26.8 | 30 | 21.2 ± 22.5 | 0.36 |
| DQB1*05 | 39 | 23.8 ± 23.7 | 1 | 27 | 0.70 |
| DQB1*03 | 17 | 33.7 ± 24.9 | 23 | 16.6 ± 19.8 | **0.02** |
| DRB1*14/DQB1*05 | 37 | 24.5 ± 24.1 | 3 | 16 ± 12.12 | 0.7 |
| DRB1*04/DQB1*03 | 7 | 37.8 ± 26.3 | 33 | 24.5 ± 23.4 | 0.09 |

**ANTIBODY TITRES : ANTI DESMOGLEIN ANTIBODIES AND SERUM IMMUNOGLOBULIN G4**

Of the 42 pemphigus vulgaris patients with active disease, immunoglobulin G4 antibody levels was done in 38 patients.

IgG4 levels in the pemphigus vulgaris patients ranged from 596 to 3199 mg/L with mean titre of 1114.4 ± 576.01 mg/L.

The serum IgG4 levels positively correlated with the Anti-Dsg3 antibody levels in patients with pemphigus vulgaris with correlation coefficient R = 0.407 and this correlation was significant (p = 0.01) ***(Figure 9).***

Although serum IgG4 levels showed a positive correlation with anti-Dsg1 titres in patients with pemphigus vulgaris, it was not significant (p = 0.06).***(Figure 10)***

Similarly the correlation between PDAI score and serum IgG4 levels showed a positive correlation (R= 0.19) but this was not significant (p = 0.25). ***(Figure 11)***

**Figure 9: Correlation of serum IgG4 with anti-Dsg3 in patients with pemphigus vulgaris**

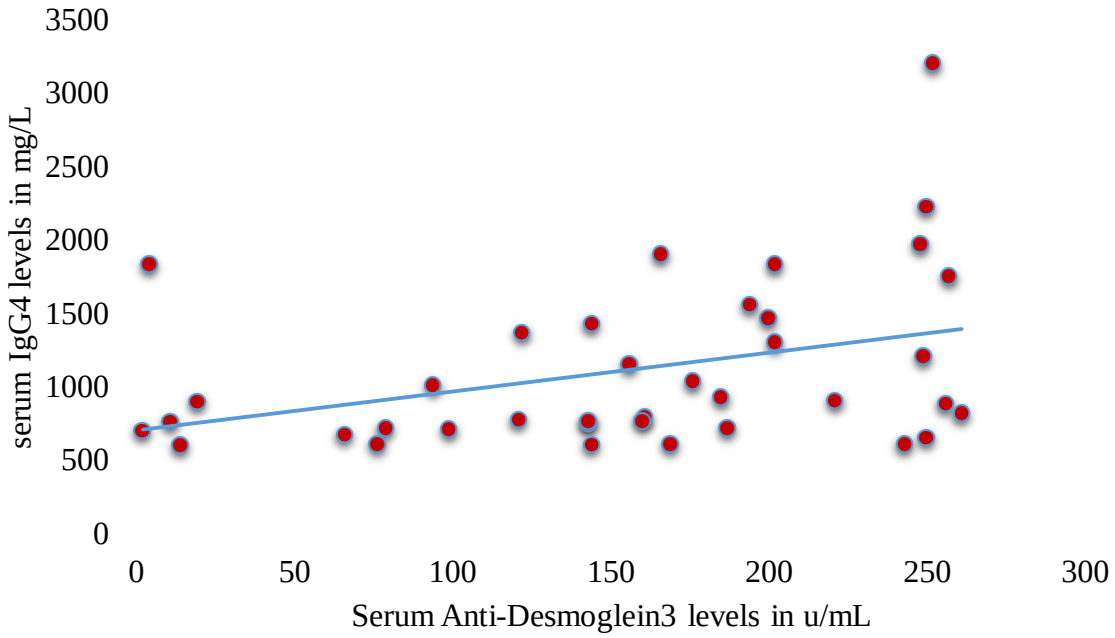
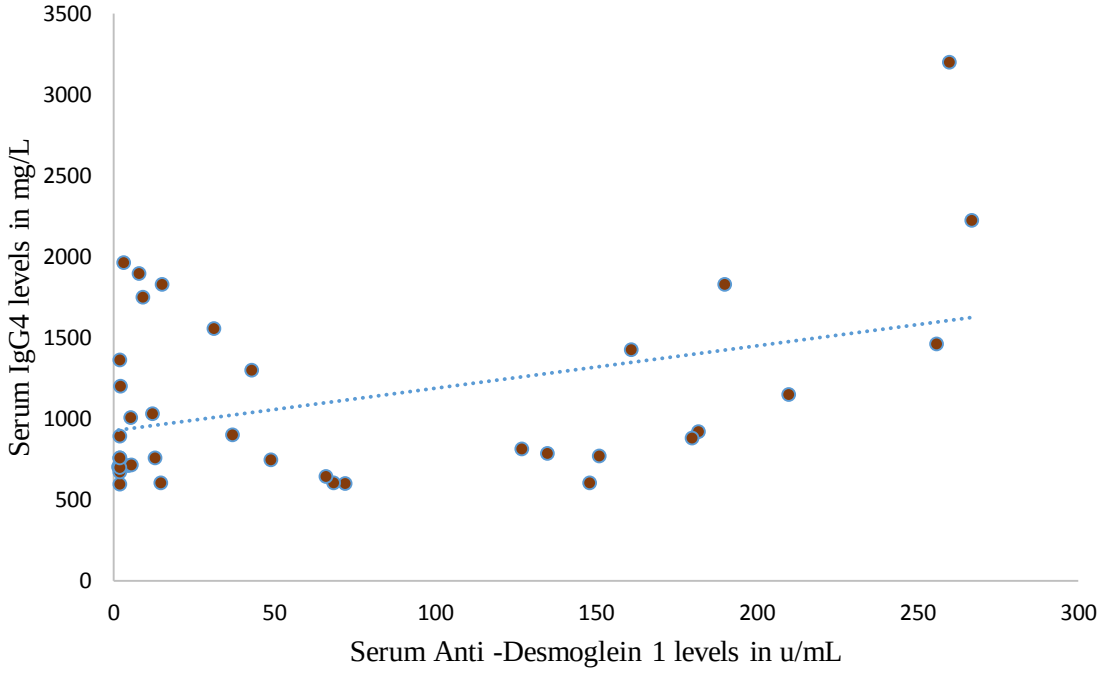
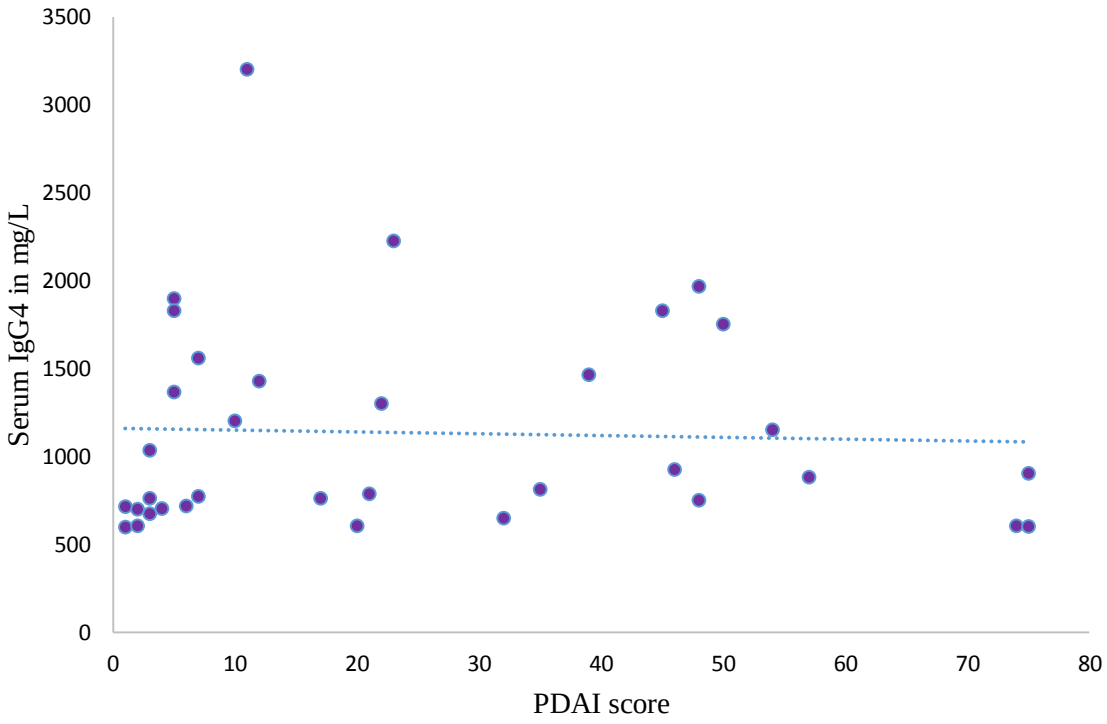


**Figure 10: Correlation of serum IgG4 with anti-desmoglein 1 in patients with pemphigus vulgaris**



Correlation of total PDAI scores with serum IgG4 in the patients with pemphigus vulgaris was not significant. ***(Figure 11)*** Although positive correlation was present (R = 0.19), it was not significant ( p = 0.25).

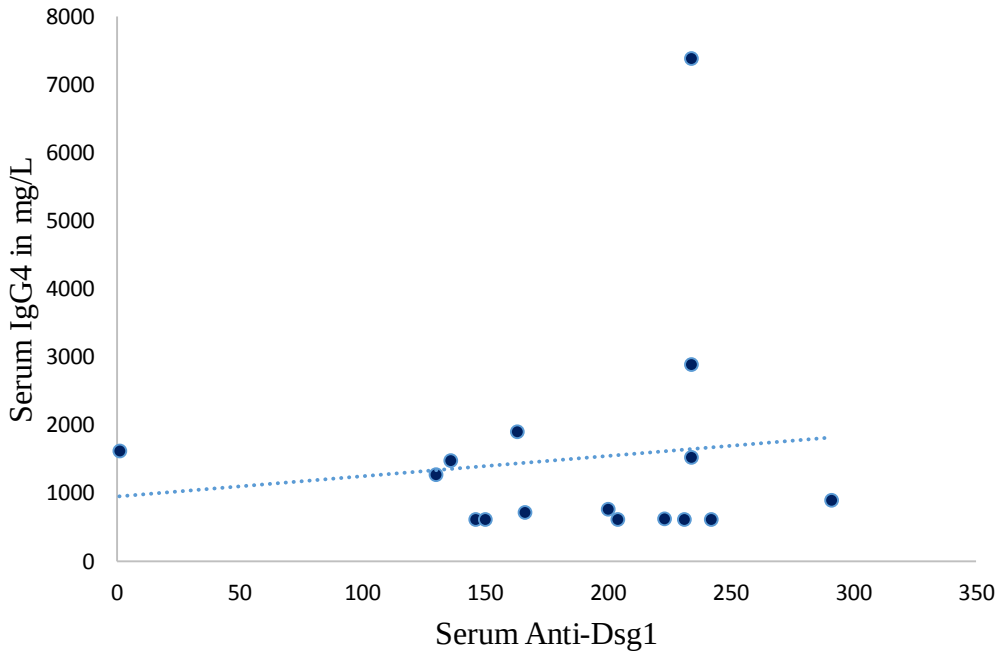
**Figure 11: Correlation of PDAI score with serum IgG4 in patients with pemphigus vulgaris**



In the pemphigus foliaceus patients IgG 4 level was available for 17/18 patients. Serum IgG4 levels ranged from a minimum level of 605 mg/L to a maximum value of 7378 mg/L. The mean IgG4 level was 1471.14 ± 1644.32 mg/L

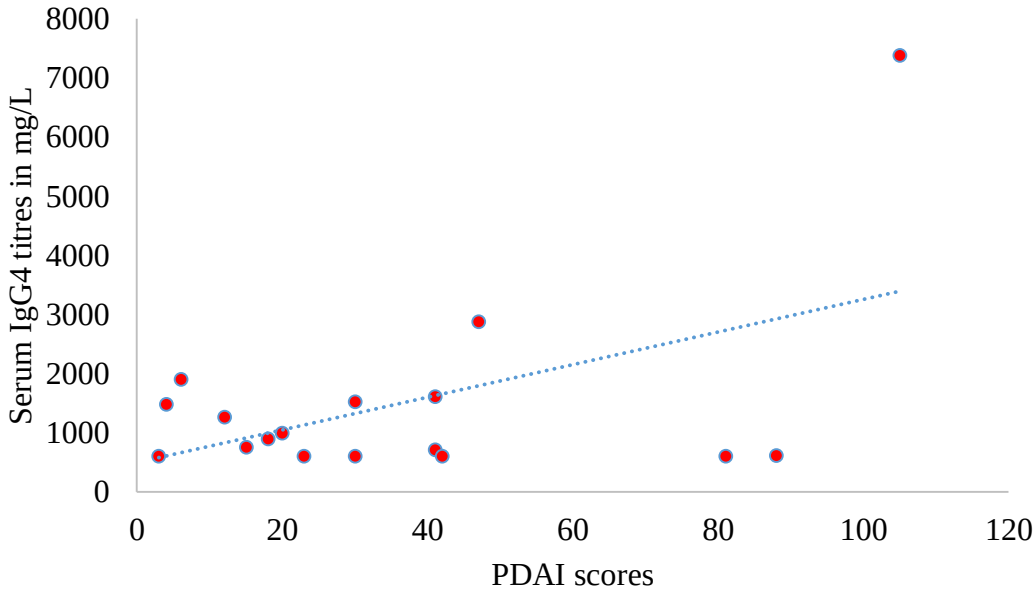
The IgG4 levels had a poor correlation with anti-Dsg1 levels in patients with pemphigus foliaceus. (R=0.006, p = 0.98) ***(Figure 12)***

**Figure 12: Correlation between IgG4 levels and anti-Dsg 1 in patients with pemphigus foliaceus**



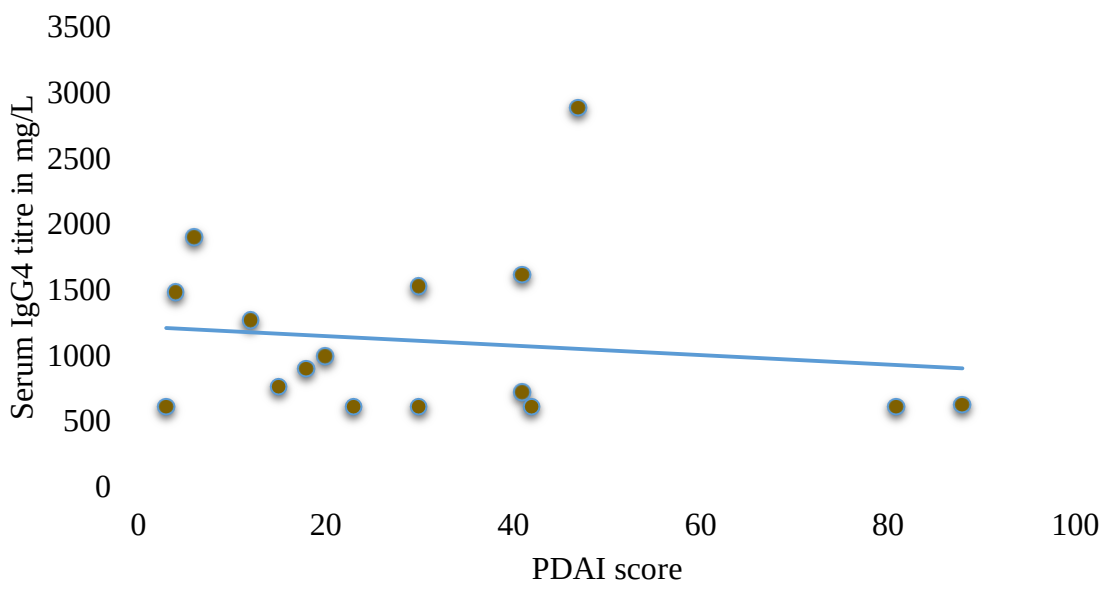
A positive correlation between disease severity (PDAI score) and serum IgG4 antibodies was demonstrated in pemphigus foliaceus patients ***(Figure 13).*** (Correlation coefficient R= 0.25, p = 0.03).

**Figure 13: Correlation of PDAI scores with serum IgG4 in patients with pemphigus foliaceus**



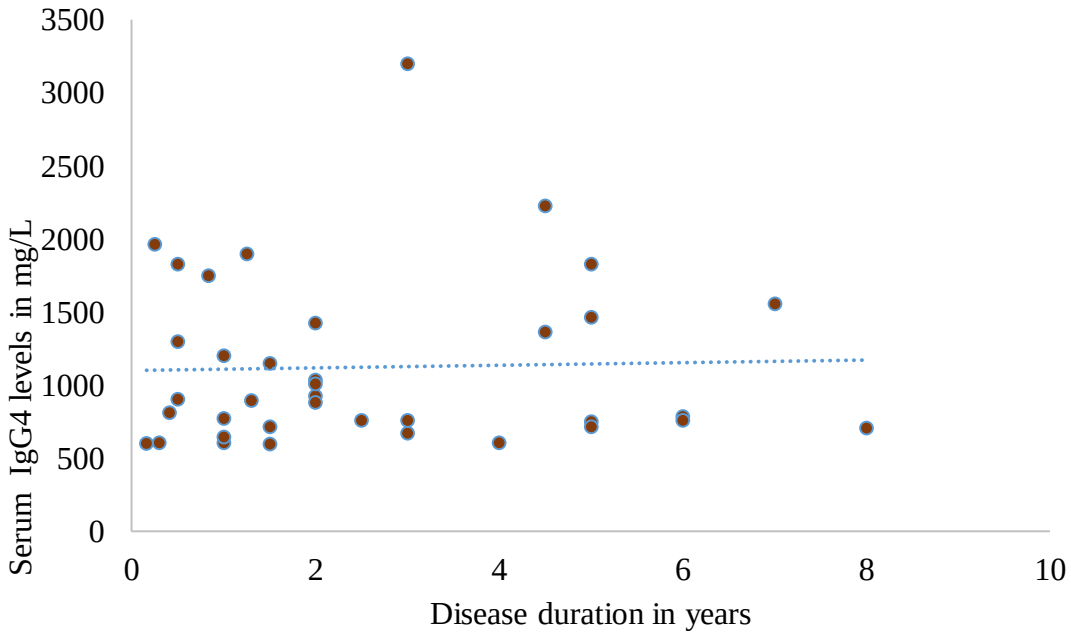
However there was one extreme value of IgG4 of 7378 mg/L. On exclusion of this value the PDAI scores did not correlate with the IgG4 levels in the pemphigus foliaceus patients. (R= - 0.16, and p = 0.54). ***(Figure 14)***

**Figure 14: Correlation of PDAI scores with serum IgG4 excluding the extreme values in pemphigus foliaceus.**



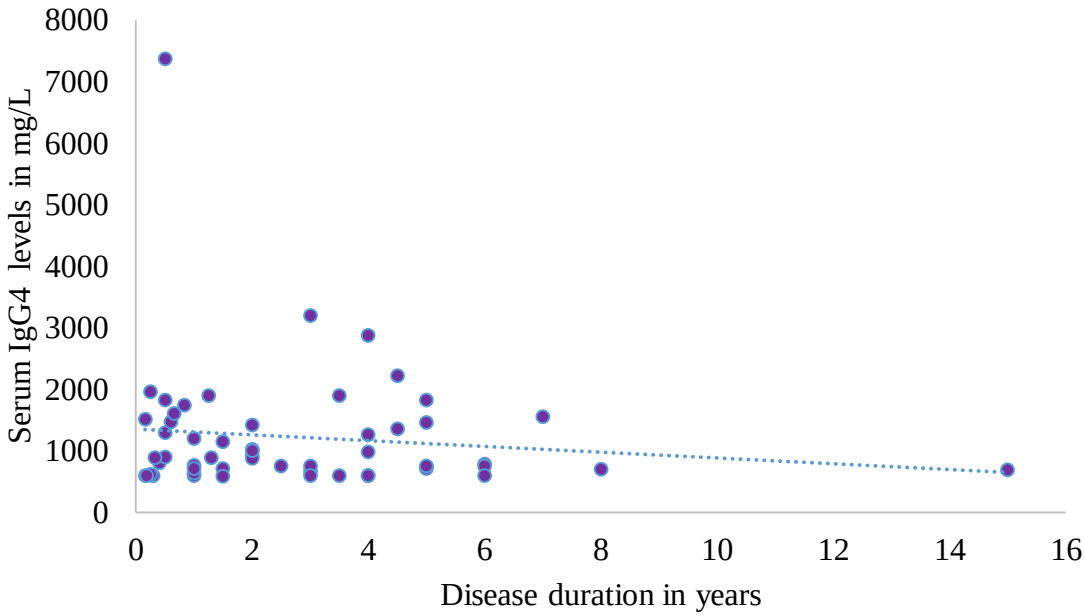
The correlation of serum IgG4 levels with disease duration in both the groups combined, pemphigus foliaceus and vulgaris showed a positive correlation but it was not of statistical significance (R = 0.032 and p = 0.8) Assessed individually, the serum IgG4 levels in relation to disease duration also showed only a weak positive correlation in pemphigus vulgaris (R is 0.032, p = 0.95). ***(figure 15)***

**Figure 15: Correlation between duration of disease and serum IgG4 in pemphigus vulgaris patients**



There was a negative correlation of disease duration with serum IgG4 seen in foliaceus group. (R= -0.144, p =0.58) ***(figure 16)***

**Figure 16: Correlation between disease duration and serum IgG4 in pemphigus foliaceus patients**



On exclusion of two extreme variables (duration -15 years & IgG4 =7378 mg/L) , the value of R was positive, R= 0.011, but this was not significant ( p = 0.93) as represented in the scatter plot in ***figure 17***.

**Figure 17: Correlation between the duration of disease and IgG4 in pemphigus foliaceus**

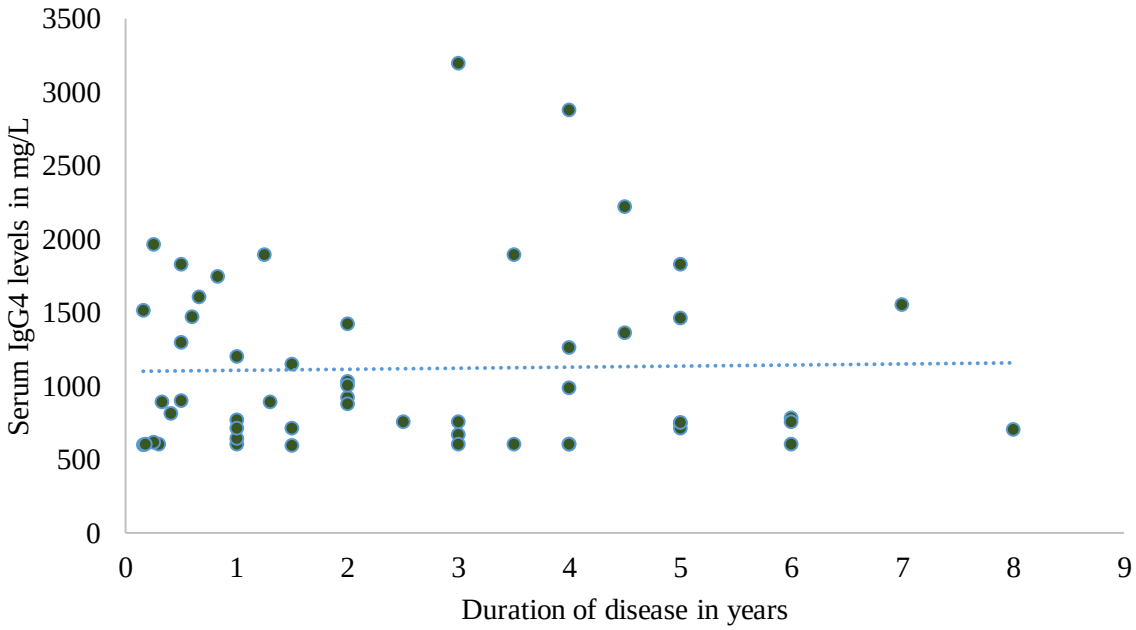


Figure 18: Flaccid bullae and moist erosions in a patient with pemphigus vulgaris

Figure 19: Erosions on the lips in a case of pemphigus vulgaris

Figure 20: Erosions on the labial mucosa in young female with pemphigus vulgaris

Figure 21: Vesicular and bullous lesions in a case of pemphigus vulgaris

Figure 22: Paronychia in a case of pemphigus vulgaris

Figure 23: Healing erosions with marginal activity and post-inflammatory hyperpigmented macules in an adult with pemphigus vulgaris

Figure 24: Eroded moist plaques in flexural region in case of pemphigus vegetans

Figure 25: Flaccid bulla and superficial crusted erosions in a young adult with pemphigus foliaceus

Figure 26: 32 year old lady with erythroderma due to pemphigus foliaceus

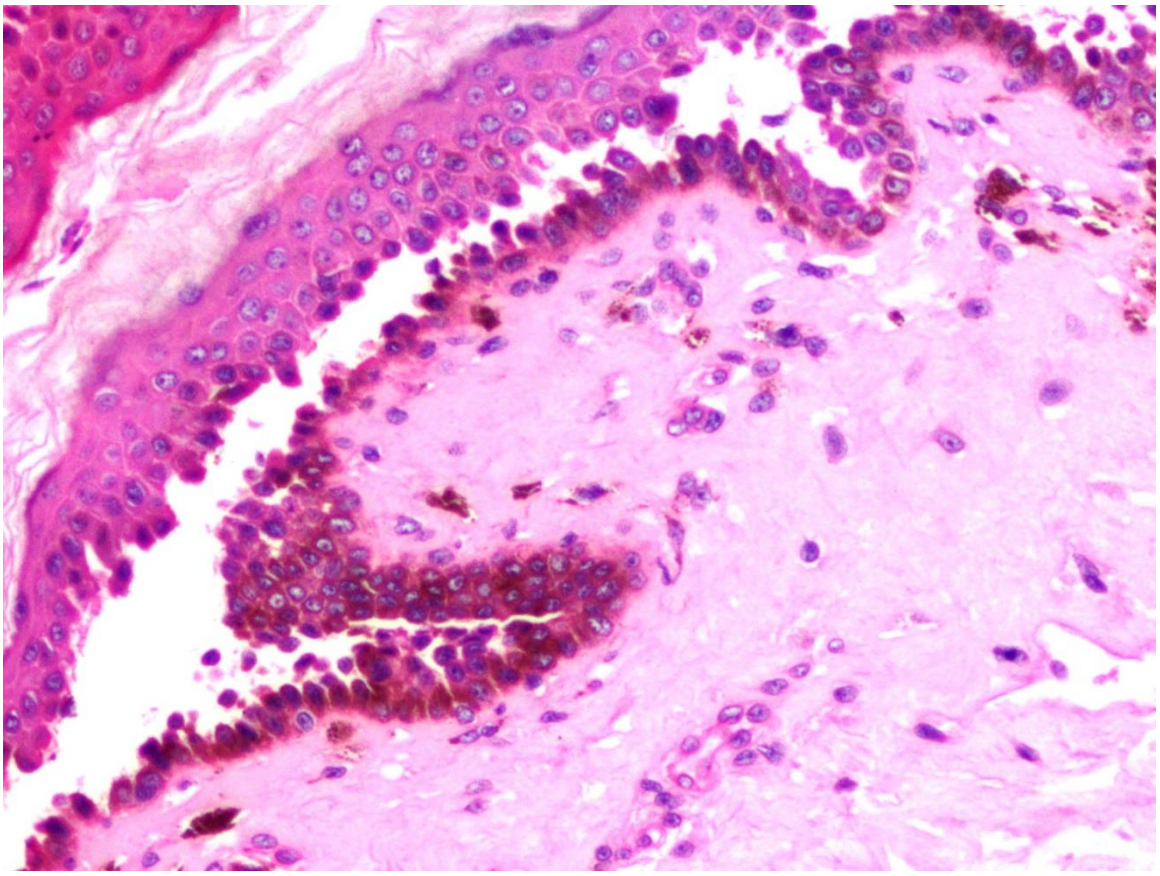


Figure 27: Suprabasal bulla with acantholytic cells in blister cavity in pemphigus vulgaris

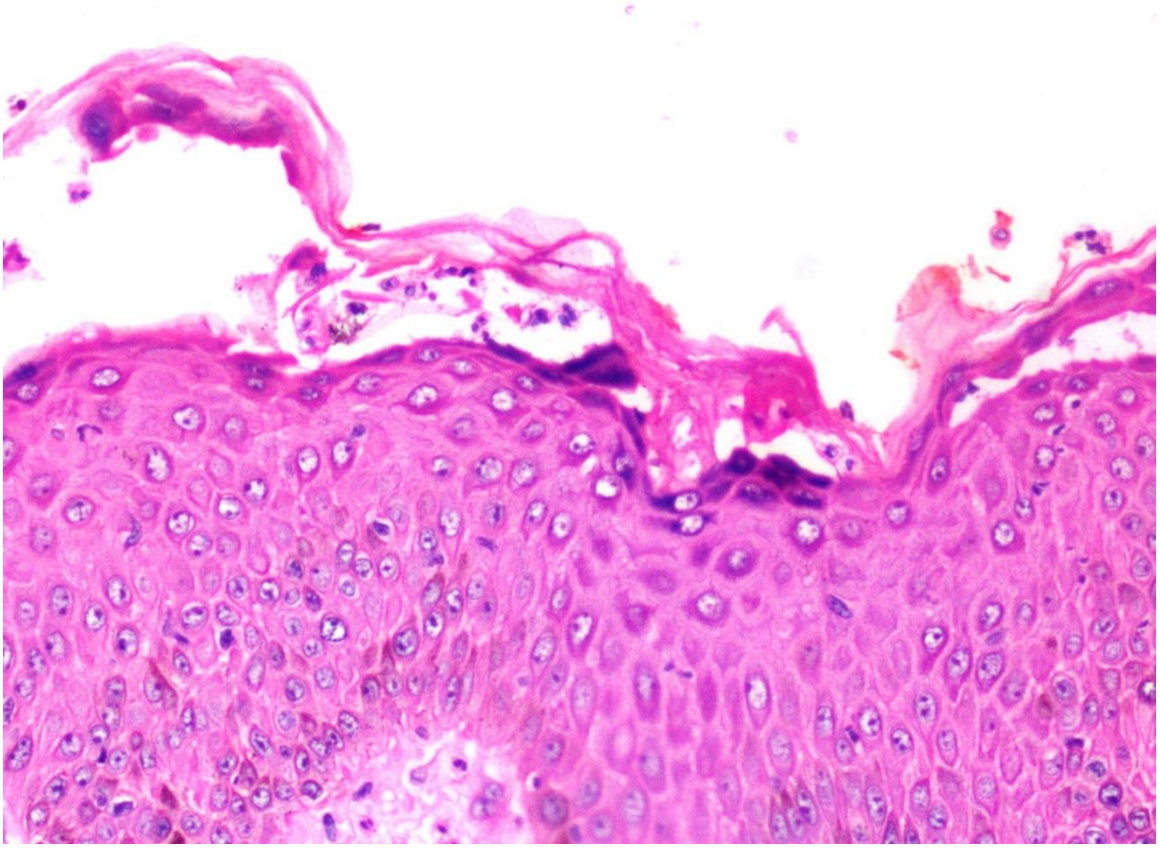


Figure 28: Subcorneal bulla with acantholytic cells in the granular layer in case of pemphigus foliaceus

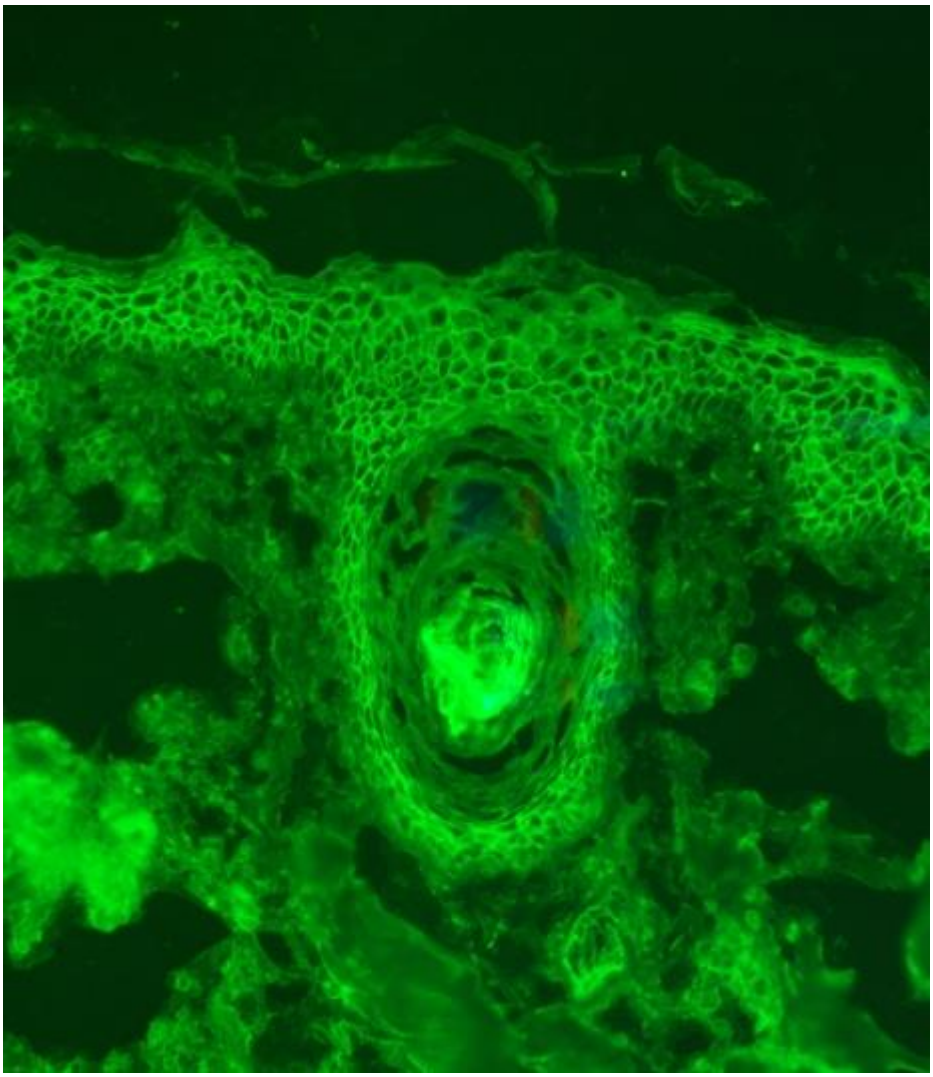


Figure 29: Intercellular fish-net pattern of IgG deposits in DIF of perilesional skin in pemphigus

# DISCUSSION

Pemphigus is an acquired immunobullous disease involving the skin and the mucosa. The pathogenesis of the disease has been extensively studied. Autoimmunity is thought to play an important role in the pathogenesis of pemphigus. This has been supported by the demonstration of tissue bound and circulating autoantibodies in patients with pemphigus. These autoantibodies are directed against the intercellular adhesion proteins. Genetic predisposition also plays a significant role in the development of the disease. The role of genes in the predisposition to pemphigus has been repeatedly reinforced by the demonstration of significantly higher frequency of occurrence of MHC class II genes in patients with pemphigus as compared to healthy individuals.

The MHC class II genes, mainly the HLA DRB1*04, DRB1*14, DQB1*03, DQB1*05 genes have been seen in patients with pemphigus vulgaris in studies from different parts of the world. Until now there have been 3 studies that have included patients of Indian origin to assess the HLA types in pemphigus, of which only 1 study was done in India. These studies have shown a higher frequency of DRB1*14 genes and DQB*05. Studies from the Western-European population have demonstrated higher frequency of DRB1*04 and DQB1*03. We undertook the present study to determine the HLA DR and DQ types that are commonly seen in patients with pemphigus in India.

Autoantibodies in pemphigus are important in the pathogenesis of the disease. IgG antibodies target the intercellular adhesion proteins and prevent their normal functioning thereby leading to acantholysis. IgG1 and IgG4 are the main subclass of antibodies that are of importance in pemphigus. It has been seen that IgG4 antibodies are the main pathogenic

antibodies and are present in high amounts during early disease activity. In this study we attempted to correlate the disease activity as measured by Pemphigus Disease Area Index with serum total IgG4 level.

Seventy two patients of pemphigus were included in our study. The youngest patient in our study was 12 years of age. There were a total of 4 patients under the age of 20 years. Juvenile pemphigus is relatively rare, but pemphigus vulgaris has also been reported at three and half years of age and 2 years of age, however the clinical details of the latter were not available.(84,85)

Among the pemphigus vulgaris 48% had disease onset below the age of 40 years. This confirms the reports of earlier onset of pemphigus in India as compared to the European population where the onset of pemphigus is usually in the $5^{th}$ decade.(46) The oldest patient had onset of disease at the age of 68 years. In Mahajan et al's study the oldest patient reported was 98 years of age.(80) In both the pemphigus vulgaris and foliaceus group of patients taken together the gender distribution showed an equal prevalence in females and males with male to female ratio 1:1. This is contrary to other Indian studies where female predominance ranging from 1.16 to 1.8: 1 has been reported. However when the two groups were analyzed separately we found female predominance in patients in pemphigus vulgaris with male : female ratio of 1:1.45. A significant male predominance was seen in the foliaceus group with M:F ratio of 3.5:1. As the sample of pemphigus foliaceus patients was smaller and only patients with active disease were recruited in the study for the purpose of correlating IgG4 with disease activity, it may not reflect the true demography of pemphigus

foliaceus. The comparison of demographic data between our study and other studies is represented in ***table 15***.

**Table 15- Comparison of demographic data between our study and other studies in literature.**

| **Parameter** | **Present study** | **Mahajan VK et al. (80)** | **Kandan S et al(19)** | **Wilson C et al(46)** | |
|---|---|---|---|---|---|
| Total no. of patients in study | 72 | 54 | 65 | 50 | 12 |
| Type of study | Prospective | Retrospective | Prospective + retrospective | Retrospective | Retrospective |
| Year of study | 2013-2014 | 1990-2002 | 2001-2008 | 1994 | 1994 |
| Ethnicity | Indian | North Indian | South Indian | North Indian | European |
| Age at onset of disease in years (range) | 43.2 (12-68) | N.A.(10-95)* | 44.6 (14-73) | 41.2 (15 -65) | 52.7 (31-79) |
| Male:Female Ratio | 1:1 | 1:1.16 | 1:1.82 | 1:1.04 | 1:1 |
| No of pemphigus vulgaris (percentage) | 51 (70.83%) | 44 (81.48%) | 49 (75.4%) | 43 | 8 |
| No. of pemphigus foliaceus (percentage | 18 (25%) | 8 (14.81%) | 11 (16.92%) | 4 | 9 |
| No. of pemphigus vegetans | 3 (4.16%) | 1 | 3 (4.6%) | 2 | 2 |
| Other types recorded | nil | 1 | 2 (2.1 %) | 1 | 1 |

The disease duration on an average was 2.81 years (2.9 years in patients with pemphigus vulgaris and 2.4 years in foliaceus patients). The longest duration of disease noted in our study was 15 years. Pemphigus is known to have a chronic course. Activity of disease reduces with time and remissions may be achieved with appropriate steroid and immunosuppressive therapy. Relapses usually occur within 2 years of the diagnosis.(86)

There were 54 pemphigus vulgaris patients (75%) and 18 patients of the foliaceus type (25%). This is similar to other studies reported from India where pemphigus vulgaris has been reported to constitute 72 to 95% of all cases of pemphigus.(87) Wilson et al in their study found that there were equal number of pemphigus foliaceus and vulgaris cases in the population from UK, while the population from New Delhi had 43 vulgaris cases out of the 50 pemphigus patients.(46)

The onset of disease in patients with pemphigus vulgaris was the oral mucosa in majority (81.48%) of the patients. This percentage is higher than Gupta et al's study where in 65% of cases mucosal lesions preceded the development of cutaneous lesions. In 2 other studies from India 50% cases had oral involvement as the primary manifestation. The duration between mucosal involvement to the development of cutaneous lesions can vary between 6-9 months.(80,88) Wilson et al reported that all patients of pemphigus vulgaris had eventually developed oral lesions.(46)

Of 42 patients with active disease, oral mucosal involvement was seen in 83.3% of patients and skin was involved in 61.1% of patients at the time of recruitment in the study. Patients with oral erosions more often present with pain and those with skin lesions often present with pruritus. Pruritus was reported in 18% of pemphigus vulgaris cases in a study of 75 cases

of pemphigus done by Vora et al. Pruritus was present in 100% of the pemphigus foliaceus patients in their study.(74)

The history of photosensitivity was present in 11.1% of patients of pemphigus vulgaris and 16.6% of pemphigus foliaceus. Photosensitivity is more often reported with pemphigus foliaceus and exacerbation of the disease with UV exposure was reported in a case-series.(89) In most studies on pemphigus the diagnosis of pemphigus erythematosus has been a clinical one without the DIF features to support it. The direct immunofluorescence features in 7 out of these 9 patients was not suggestive of pemphigus erythematosus. ANA was done in 5 of the 9 patients and was negative in all the 5. ANA in pemphigus vulgaris patients has been reported positive in 26.7% of cases.(90)

The history of a possible drug trigger was present in a single patient of pemphigus vulgaris. The suspected drug was enalapril. The thiol group of drugs are known to trigger pemphigus.(67) Drug induced pemphigus has not been commonly reported in Indian studies, probably due to the low index of suspicion. Also drug induced pemphigus is difficult to diagnose in the absence of specific diagnostic test.

The well known associations of pemphigus include myasthenia gravis, thymoma, lupus erythematosus and neoplasia in case of paraneoplastic pemphigus.(3) In our study, 72.2% of had associated comorbidities. Hypovitaminosis D was the commonest associated comorbid condition, which was present in 27 patients (35.5%). This association is probably fortuitous and may be attributed to the generally high prevalence of vitamin D deficiency in India.(91) Diabetes mellitus type -2 was seen in 33.3% patients and hypertension in 18 patients (25%), while osteopenia and osteoporosis was present in 9.7% cases. Hypothyroidism was present in

3 patients. Other conditions that were a chance occurrence, seen in one patient each were hepatitis C, HIV, bronchial asthma, atopy and adjustment disorder. Single patient at the time of inclusion had retroperitoneal fibrosis, and six months later was found to have non-Hodgkin's lymphoma, however the clinical features, histopathology and direct immunofluorescence findings were consistent with pemphigus vulgaris type, thus paraneoplastic pemphigus was ruled out. Gupta et al reported autoimmune conditions in association with pemphigus vulgaris in 20.5% of cases. Thyroid dysfunction was reported in 45% of their study population, the other autoimmune associations being rheumatoid arthritis, alopecia areata, type 1 diabetes mellitus, and one patient each with lupus erythematosus, myasthenia gravis and vitiligo.(14)

Ninety-three percent of patients in our study were on treatment with steroids prior to inclusion in the study. Steroid therapy was most often instituted orally. Twelve patients had received dexamethasone- cyclophosphamide pulse therapy with inter-pulse oral steroids.

We found the most common steroid sparing adjuvant used was azathioprine (50.7%). It is a well established adjuvant with equal efficacy to mycophenolate mofetil (MMF), but provides relatively faster disease control.(92) Only 2 patients had received MMF. Mycophenolate mofetil, is considered to have a better side effect profile, and has been considered the first choice for adjuvant in pemphigus in patients with mild to moderate disease severity.(93) Cyclophosphamide was used in 17.8% of patients. Daily administration of cyclophosphamide is said to have the best disease control but is also associated with higher cumulative toxicity.(94) Methotrexate is infrequently used in treatment of pemphigus, but has proven efficacious in improving disease in over 90% patients.(95) In our study 5 patients had received methotrexate in the past. One of these 5, a patient of pemphigus vulgaris, had

been on methotrexate for 2 years and had good control of the disease with anti-desmoglein antibody levels of <2u/mL.

Oral mucosal involvement was seen in 83.3% cases. This is in concordance with other studies where the involvement of mucosa has been reported in 75% , 78.3% to 97%.(14,19,80) Three cases were mucosal predominant pemphigus (5.5%), and there was one patient with cutaneous pemphigus vulgaris, 92.6% were mucocutaneous type of pemphigus vulgaris. Mucocutaneous pemphigus is the most common clinical type of pemphigus vulgaris. It was seen in 63.63% in Mahajan et al's study, 65.85% in Gupta et al study.(14,80) Wilson et al reported that mucosal lesions developed in all cases of pemphigus vulgaris from India as compared to 8 of 10 cases from UK.(46) Gupta et al reported that scalp was the most common site of involvement followed in frequency by lesions on the trunk.(14) In our study, trunk was the most common site of involvement 73.8%, scalp involvement was seen in 47.6%. Nail involvement in pemphigus is less common, but its presence is associated with severe disease.(96,97) It was seen in 16.6% cases in our study.

Of the pemphigus foliaceus group, one patient had erythroderma. Mahajan et al reported one case of erythrodermic pemphigus foliaceus in his study of 54 cases. Erythroderma in pemphigus is more often associated with endemic forms of pemphigus foliaceus and less frequently with non- endemic pemphigus foliaceus.(3) Although pemphigus foliaceus has a benign course as compared to vulgaris, when in erythroderma it can prove to be fatal.(98)

Nikolsky sign was positive in 18% of pemphigus vulgaris patients and 50% of foliaceus patients. Nikolsky sign is not very sensitive but is a relatively specific sign for pemphigus. Arya et al in their study on 70 pemphigus cases, reported Nikolksy sign positive in 97.2% of

vulgaris patients and 94.7% in foliaceus patients.(99) The lower rate of positive Nikolksy sign in our study is probably because patients were already on treatment prior to inclusion in the study.

The PDAI score in patients with pemphigus vulgaris ranged from 1 to75 with a mean score of 24.1. Among the pemphigus foliaceus patients the PDAI scores ranged from 3 to 88 in most patients, and maximum score of 105 in the erythrodermic patient. The mean score in foliaceus patients was 33.8. Overall the mean PDAI scores was not very high since most patients were on treatment. Mean PDAI scores in our patients was higher than that seen in Rosenbach et al's study of 15 patients of pemphigus vulgaris (24.1 versus 10.2).(82) Their study included pemphigus vulgaris patients with mild to moderate disease. Mean PDAI scores in our pemphigus vulgaris patients was lower than that seen in Chams –Davatchi et al's study on 56 pemphigus. The mean PDAI of pemphigus vulgaris patients was 53.2 in their study. This was probably because treatment naïve patients were included in their trial.(100)

Tzanck smear is an easy and rapid diagnostic test available for pemphigus. Tzanck smear was positive for acantholytic cells in 70.7 % cases in our study. Our result was comparable to Patel et al's results of 75% positivity of Tzanck smear.(101) Leena et al reported 100% positivity of Tzanck smear in their clinicopathological case series.(102) Biopsy was confirmatory of pemphigus vulgaris in 88.4% cases, while in the remaining cases the typical features of pemphigus vulgaris was not seen. This was probably because biopsy was not from an early vesicular lesion. Leena et al reported typical suprabasal bulla with acantholytic cells in 94.4% cases. In a study done by Arundhati et al on clinical histopathological and immunofluorescence features in immunobullous diseases they reported typical histopathological features in 26 of the 36 cases in pemphigus vulgaris and 4 of the 10 cases in

pemphigus foliaceus.(84) In our study histopathology was contributory of pemphigus foliaceus in 100% cases in whom it was done.

Direct immunofluorescence was positive for IgG in 100% cases of pemphigus foliaceus, however C3 was present only in 44% of the cases. Whereas in the vulgaris group IgG was detected in 40 cases (85%) and complements in 33 cases 70%. Arundhati et al found DIF to be positive in 92% of the vulgaris patients and 75% of foliaceus patients. Overall direct immunofluorescence was negative in 34% of their cases. They emphasized the fact that none of these investigations done alone is diagnostic in each and every disease, therefore the diagnosis of immunobullous disease is made by combination of clinical features, Tzanck smear, histopathology and immunofluorescence.(84) The reason for negativity of DIF may be a delay in tissue processing after sampling if it is not preserved in appropriate media. The other reason may be effect of initiation of immunosuppressant.

ELISA anti-desmoglein antibodies are specific for the disease, and are also found to correlate with the disease phenotype. In our study the Anti-Dsg 3 antibody titres correlated positively with the PDAI scores in patients with pemphigus vulgaris. Similarly the anti-Dsg1 antibody titres showed a positive correlation with PDAI scores of pemphigus foliaceus patients. This is similar to study done by Kumar et al, where desmoglein ELISA titres was correlated with severity of skin disease in 38 vulgaris and 6 foliaceus patients. They found that high anti-Dsg3 titres correlated with severe mucosal involvement in pemphigus vulgaris and anti-Dsg1 titres correlated well the severe skin involvement. The antibody titres, however, remained high even 6 months after resolution of skin lesions.(60) The correlation between desmoglein antibodies and severity was also attempted by Harman et al's study on 88 pemphigus vulgaris and 24 foliaceus cases. Harman et al demonstrated 34% chance of having higher skin

severity score and 25% chance of higher mucosal severity score with 10 unit rise in anti-Dsg3 and anti-Dsg1 antibodies respectively.(81)

In our study, the serum total immunoglobulin G4 antibody titres in the 38 pemphigus vulgaris patients did not correlate with the disease severity scores. On the other hand in the pemphigus foliaceus patients the serum IgG 4 titres had a positive correlation with PDAI scores. This result however was affected by an extreme value of IgG4 (7378mg/L) which was associated with the erythrodermic patient. On excluding this value there was no correlation between disease severity and the antibody titres. The correlation of disease severity scores with total IgG4 has not been studied before. In view of the positive correlation of anti-desmoglein antibodies with PDAI in this study we can infer that desmoglein specific IgG4 may show a good correlation with disease activity. Since the desmoglein specific IgG4 was not available at our Institute we tested for total IgG4 levels. The other possible reason for this may be that most of the patients had already been initiated on immunosuppresant. As Dhandha et al has demonstrated the IgG4 antibodies fall more rapidly when IgG1 antibodies when the disease remits.(63)

We did not find a positive correlation between disease duration and serum total IgG4 levels in our patients. Although a positive correlation was noted in pemphigus foliaceus patients on exclusion of extreme value of serum IgG4 titre, the correlation was not significant to draw any conclusions. This result was contrary to Dhandha et al's finding of higher IgG4 levels in patients with longer disease duration. Dhandha et al proposed that serum anti-desmoglein IgG4 levels rise with longer duration of disease.(63)

**Table 16-Baseline characteristics of studies on HLA types in Pemphigus**

| Study | Ethnicity | Diagnostic criteria | No. of cases | No. of controls | Genotyping method |
|---|---|---|---|---|---|
| Present study | Indian | Clinical + HPE/ DIF/ Dsg ELISA | 50 | 50 (UR) | PCR SSOP |
| Delgado et al(34) | Indian | Clinical + IIF | 39 | 89 (R ) | PCR SSOP |
| Delgado et al(39) | Pakistani | Clinical +IIF | 19 | 13(UR) | PCR SSOP |
| | European | Clinical +IIF | 38 | 496(UR) | PCR SSOP |
| Miyagawa et al (38) | Japanese | Clinical +HPE/ DIF /IIF | 16 | 525(UR) | PCR RFLP |
| Saha et al(6) | Caucasian European | NA | 96 | 100(UR) | PCR SSP |
| | Indo-Asian | NA | 57 | 59(UR) | PCR SSP |
| Shams et al(31) | Iranian | Clinical + HPE/ DIF | 52 | 180(UR) | PCR SSP |

NA = data not available from study, R= related, UR=unrelated controls, PCR SSOP-Polymerase chain reaction, sequence specific oligonucleotide probe, SSP-sequence specific primers, RFLP-restriction fragment length polymorphism, IIF-indirect immunofluorescence, HPE-histopathological examination, DIF- direct immunofluorescence.

**Table 17- Comparison of the predominant HLA DRB allleles (allele frequency in % ) between our study and other studies on pemphigus**

| Study title | DRB1*14 | | | DRB1*04 | | | DRB1*15 | | | DRB1*11 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **case** | **con** | **p** | **case** | **con** | **p** | **case** | **con** | **p** | **case** | **con** | **p** |
| Present study | 94 | 36 | **<0.001** | 26 | 16 | 0.2 | 14 | 32 | 0.01[a] | 4 | 18 | 0.02[a] |
| Delgado et al(34) | 45.9 | 13.5 | <0.001 | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Delgado et al(39) | 55.3 | 23.1 | 0.01 | 10.5 | 34.6 | NS | NA | NA | NA | 0 | 7.7 | NS |
| | 36.8 | 4.2 | $<10^{-6}$ | 23.7 | 15.3 | NS | NA | NA | NA | 5.3 | 11.1 | NS |
| Miyagawa et al(38) | 27.6 | 6.8 | <0.05 | 43.8 | 6.7 | <0.05 | NA | NA | NA | NA | NA | NA |
| Saha et al (6) | 46.5 | 11.9 | <0.001 | 14 | 0.8 | <0.001 | 4.4 | 21.2 | <0.001[a] | NA | NA | NA |
| | 22.9 | 4.0 | <0.01 | 37 | 14 | <0.01 | 4.2 | 12.5 | <0.01[a] | NA | NA | NA |
| Shams et al(31) | 25 | 5 | <0.01 | 36.5 | 9.2 | <0.01 | NA | NA | NA | 13.5 | 24.7 | 0.01[a] |

NA = data not available NS=not significant, but p value not mentioned in study

Con=controls [a]significantly high in controls.

As presented in the ***table 17*** the HLA DRB1*14 is significantly higher in pemphigus patients as compared to controls in most ethnic groups. In our study HLA DRB1*14 allele frequency was significantly higher in pemphigus patients (47 of 50cases) as compared to that in controls (17 out of 50) (p value <0.001). Nine patients of the 47 were homozygous for this allele.

Our results were consistent with Delgado et al's study on Indian patients of pemphigus vulgaris where HLA DRB1*14 was the most frequently seen DRB allele.(34) The association of HLA DRB1*14 with pemphigus was also supported by Yan et al's meta-analysis that included 18 studies from different parts of the world.(30) Saha et al's study population was divided into White Europeans (Caucasians) and Indo-Asians.(6) Their study also confirmed that DRB1*14 is more often associated with pemphigus patients of Indo-Asian origin as compared to their Caucasian population. However the percentage of patients that were of Indian origin was not mentioned.

The DRB1*04 allele was found to be slightly increased in our patients (26%) as compared to in normal individuals (16%), however this difference was not statistically significant. This allele is seen more often in the Ashkenazi Jewish and in most Non-Jewish Caucasian population.(73) Specifically, DRB1*0402 is the allele that is identified with susceptibility to pemphigus in Ashkenazi Jews.(33) DRB1*04 was not reported in Delgado et al in his study on Indian pemphigus patients.(34) In Saha et al's study DRB1*04 was found to be significantly increased in the Indo-Asian population. This may have been due to the varied ethnicity and this study cannot be taken to truly reflect the genotype of pemphigus patients of Indian race.(6) The meta-analysis by Yan et al also reported significant association of DRB1*04 with pemphigus patients from different ethnic populations. DRB1*0402, apart

from the Jewish population was seen to be associated with pemphigus patients of European and Iranian origin, whereas DRB1*0403 and DRB1*0404 were associated with the Japanese population.(30)

The increased frequency of HLA DRB1*15 in controls (32%) versus pemphigus patients (14%) that was seen in our study is also noted in Saha et al' s study, as seen in both their study groups. The negative association of the allele DRB1*15 is confirmed by the meta-analysis by Yan et al.(30) The other allele that was increased in controls as compared to patients in our study population was DRB1*11 ($p = 0.02$). This is a relatively rare negative association, but has also been noticed in study by Shams et al on patients of Iranian origin.(31) Yan et al's meta-analysis found sufficient evidence for other alleles that have been negatively associated with pemphigus and therefore thought to have a protective role in pemphigus. These include DRB1*07 and DRB1*03.(30) Of these DRB1*07 was found to be slightly increased in controls (14/50) as compared to patients (8/50) in our study but not in percentages that would reach significance level. DRB1*03 was noted in only in 3 controls and 1 pemphigus patient in our study. The frequency of DRB1*15 and DRB1*11 which were increased in our control population was not found in Delgado et al's study.(34)

In a different study by Delgado et al HLA types in pemphigus patients from Pakistan and a set of Non –Jewish Caucasian population was studied.(39) This study also reported DRB1*14 to be significantly higher in pemphigus patients from both ethnicities. Interestingly their study, similar to our study, did not show a significant increase in DRB1*04 in the Pakistani or the Caucasian population.

As expected from other Asian countries, the study on Japanese population has also shown results similar to our study with respect to DRB1*14. In addition to DRB1*14, DRB1*04 was also significantly high in the Japanese pemphigus patients.(38)

**Table 18- Comparison of HLA DQB1 alleles (allele frequencies in %) between present study and other studies on pemphigus**

| Study title | DQB1*05 | | | DQB1*03 | | | DQB1*06 | | |
|---|---|---|---|---|---|---|---|---|---|
| | case | con | p | case | con | p | case | con | p |
| Present study | 94 | 48 | <**0.001** | 48 | 50 | 0.86 | 16 | 38 | 0.02[a] |
| Delgado et al(34) | 48.6 | 17.9 | <0.001 | 16.2 | 7.9 | NS | NA | NA | NA |
| Delgado et al(39) | 57.9 | 26.9 | 0.02 | 7.9 | 15.4 | NS | 5.2 | 7.6 | NS |
| | 36.8 | 4.3 | $<10^{-6}$ | 23.7 | 9.2 | NS | 5.3 | 4.8 | NS |
| Saha et al (6) | 32.8 | 9 | <0.001 | 22.4 | 4.0 | <0.001 | 3.1 | 11.5 | 0.002[a] |
| | 47.4 | 12.7 | <0.001 | 15.8 | 6.8 | 0.049 | 4.4 | 15.3 | 0.011[a] |
| Miyagawa et al(38) | 31.2 | 4.8 | <0.05 | 50 | 17.1 | <0.05 | NA | NA | NA |
| Shams et al(31) | 18.25 | 2.5 | <0.001 | 32.7 | 5 | <0.001 | 0.95 | 10.3 | 0.004 |

Con – controls, NA=data not available from study, NS =not significant, p value not mentioned in study, [a]significantly high in controls.

Among the different DQB alleles we found significant increase in DQB1*05 in our pemphigus patients (94% cases and 48% controls) (p value <0.001).***(Table 18)*** Ten patients showed homozygosity for the presence of DQB1*05. Our result is consistent with other studies that have looked at DQB alleles in pemphigus. (5,31,34,38,39) Delgado et al's study on Indian patients found significant association of DQB1*05 in pemphigus patients.(34) In the study on Pakistani and Caucasian patients by the same author, DQB1*05 was reported highly significant in the Caucasian population and in lower levels of significance (p value =0.02) in the Pakistani population.(39) HLA DQB1*03 is the other allele that is reported to have a strong association with pemphigus patients. In our study DQB1*03 was found in nearly equal frequency in patients (24/50) and controls ( 25/50). Our results with respect to this HLA type was similar to the result of Indo-Asian population in Saha et al's study. They reported the allele in higher frequency in the Caucasian population, but not in Indo-Asians.(6) The study on Iranian as well Japanese population has also provided evidence for the association of DQB1*03 with pemphigus.(31,36)

We also noted higher frequency of DQB1*06 in controls (38%) versus patients (16%), although the strength of the difference was relatively marginal (p value = 0.02) This result was in keeping with the results of the other studies**.*(Table 18)*** Saha et al found similar negative association of DQB1*06 in both the Indo-Asian as well as the Caucasian population.(6) The same was also noted in the Iranian population.(31) However DQB1*06 was not reported from the study on Japanese patients.(38) In Delgado et al's study the representation of this allele did have significant difference between patients and controls of both the Pakistani and the Caucasian series.(39)

**Table 19- Comparison of haplotype frequency (in %) between our study and other studies on pemphigus**

| Study title | DRB1*14, DQB1*05 | | | DRB1*04, DQB1*03 | | | DRB1*15,DQB1*06 | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Cases** | **Controls** | **P** | **Cases** | **Controls** | **P** | **cases** | **controls** | **P** |
| Present study | 88 | 28 | **<0.001** | 24 | 12 | 0.13 | 10 | 30 | 0.018[a] |
| Miyagawa et al (38) | 31.2 | 4.7 | <0.01 | 43.8 | 6.7 | <0.001 | NA | NA | NA |
| Shams et al (31) | 25 | 4.4 | $<10^{-3}$ | 36.5 | 5.8 | $<10^{-3}$ | 0.95 | 6.4 | 0.02[a] |

[a]stronger significance in controls.

Haplotype analysis in our study revealed a statistically strong association for the haplotype DRB1*14 DQB1*05 with pemphigus (88% patients and 28% controls). Our result is consistent with that of other studies that have analyzed haplotypes in pemphigus patients. (5,31,38).(***Table 19)*** Delgado et al have also mentioned the occurrence of DRB1*14 and DQB1*05 together to have strong association with pemphigus patients in both of their study groups, Pakistani ($p < 10^{-6}$) and Caucasians ($p = 0.01$), however the exact percentages was not mentioned for this data.(39) This study provided evidence for the presence of autoantibodies in subjects who carried this haplotype, emphasizing its pathogenic role. They also noted the haplotype DRB1*04, DQB1*03 in 9 of the non- Jewish Europeans, although of no statistical significance.

The haplotype DRB1*04/DQB1*03 was increased in number in patients (24%) as compared to the controls (12%) in our study, but this difference failed to achieve significance levels at $p < 0.05$. This was unlike the results on Japanese and Iranian patients. It is identified as the characteristic haplotype of Ashkenazi Jews that predisposes them to higher incidence of pemphigus. The haplotypes that are probably relevant in disease causation include DQA1*0505 that occurs in association with DRB1*04 and DQB1*03. Another of the DQA allele is the DQA1*0101 which appears frequently with DRB1*14 and DQB1*05. However in our study we did not test for DQA1 alleles.

The allele DQB1*03 was associated with more severe disease as seen by higher PDAI scores in patients carrying this allele ($p = 0.02$), despite the fact that the allele frequency did not reach significance levels when compared to control population. This finding was similar to the results of Svecova et al's recent study.(104) The severity of disease was measured by ABSIS score in this study. They demonstrated a strong association between severe disease and presence of DQB1*03 allele. They also found similar association for DRB1*04. Our

study was dissimilar in that respect, patients with DRB1*04 did not have high scores of PDAI. Svecova et al's study also demonstrated an association between the DRB1*1454 with male gender, which has not be reported before. Such a difference in DR or DQ allele with respect to gender was not seen in our study.

# CONCLUSIONS

1. Pemphigus vulgaris was the most common type of pemphigus seen in our study (75%), in keeping with other studies from India. Pemphigus foliaceus was the second most frequent type of pemphigus seen in our study. Less common variants, like pemphigus vegetans was seen in 4.1%.

2. Majority (48%) of the pemphigus vulgaris patients had the disease onset at less than 40 years of age, while the average age at onset was higher in the foliaceus patients (45years).

3. Slight female predominance is seen in pemphigus vulgaris M:F ratio of 1:1.54.

4. Oral mucosa was the most common site of onset of disease in pemphigus vulgaris as reported by 81.6% patients. Pain was the most common symptom associated with it. Pruritus was the most common symptom in pemphigus foliaceus patients.

5. Vitamin D deficiency, diabetes mellitus type 2 and hypertension were the three most common associated comorbid conditions.

6. The histopathological features were typical of pemphigus vulgaris in 88.4% of pemphigus vulgaris patients and in 100% pemphigus foliaceus patients. DIF was positive in 91.5% cases of pemphigus vulgaris and in 100% of pemphigus foliaceus.

7. Ninety four percent of pemphigus patients were carriers for HLA DRB1*14 on at atleast one allele, and 18% were homozygous for it. Ninety four percent of cases had at least one DQB1*05 allele and 20% were homozygous for it. Strong statistical association was present for DRB1*14 and DQB1*05 in pemphigus patients as compared to controls (p

<0.001). It was also reflected in haplotype frequency. This was in keeping with data from other genotype studies on pemphigus patients.

8. The DQB1*03 showed significant association with severity of disease (p = 0.02), but the allele was seen only in 48% patients.

9. Anti-desmoglein 3 antibodies showed a positive correlation with PDAI scores in pemphigus vulgaris patients and anti-desmoglein 1 antibodies showed a positive correlation with PDAI scores in pemphigus foliaceus patients.

10. The serum total IgG4 titres did not significantly correlate with disease duration or PDAI scores in both pemphigus vulgaris and foliaceus patients in our study. One probable reason for this discrepancy is that we tested for serum total IgG4, not specific to desmogleins. The other reason maybe the effect of prior treatment with immunosuppressants that had reduced the antibody response.

# LIMITATIONS

Desmoglein specific IgG4 levels was not available which may have affected our results.

There were very few treatment naïve patients, therefore the antibody response and its correlation with disease severity may not reflect the true nature of disease.

We did not have HLA typing of high resolution, therefore the subtypes of DRB1, and DQB1 could not be studied.

The HLA DQA types were not studied.

# RECOMMENDATIONS

Desmoglien specific IgG4 would probably better reflect the disease severity and serial estimation of the antibody levels could be used to guide treatment in patients with pemphigus.

HLA DRB1*14 and DQB1*05 alleles are strongly associated with pemphigus and can be done in relatives of patients with pemphigus to look for susceptibility to pemphigus.

Subtype of HLA DR and DQ and other genes such as the DQA and non –HLA genes should be further studied in the Indian population for better insight to cause of disease.

# SUMMARY

**Introduction:** Pemphigus is a chronic autoimmune blistering disease that affects the skin and the mucosa. The disease is characterized by loss of adhesion between keratinocytes of the epidermis. Genetic predisposition and autoimmunity play an important role in the pathogenesis of the disease. HLA DRB1*04, DRB1*14 and DQB1*03 and DQB1*05 are reported to have a significant association with pemphigus vulgaris. The genetic predisposition varies with ethnicity. We undertook the present study to find out the HLA DR and DQ types that are seen in association with pemphigus vulgaris in India.

Antibodies against desmoglein in pemphigus are of the IgG class. Of the different subtypes, IgG4 is found to be raised in active phase of the disease whereas IgG1 remains elevated even during remission of disease. In the present study the correlation between severity of disease as assessed by PDAI in patients with pemphigus vulgaris and pemphigus foliaceus and serum IgG4 levels was also evaluated.

**Objective** : The primary objective was to compare the HLA DR and DQ types of pemphigus vulgaris patients with that of normal healthy subjects.

The secondary objective was to look at the correlation between disease severity in pemphigus vulgaris and foliaceus, measured by PDAI with serum total IgG4 levels.

**Method :** This study was a prospective case-control study done in a tertiary level hospital in India. The study period was 10 months (November 2013 to August 2014). All patients

diagnosed to have pemphigus vulgaris based on clinical, histopathological, direct immunofluorescence or serological evidence were included in the study. HLA DR and DQ typing was done by PCR-SSOP method. HLA types for controls was chosen from healthy renal transplant donors. Pemphigus foliaceus patients with active disease were included to study the secondary objective. Serum IgG4 was done in all patients with active disease. Chi-square test was used to analyze the frequencies of various HLA DR and DQ types between cases and controls. Spearman Rho was used to correlate the serum IgG4 levels and PDAI scores. Mann-Whitney U test was used to look for the association between HLA types and disease severity.

**Results :** The study included 72 patients, 54 (75%) had pemphigus vulgaris and 18 (25%) had pemphigus foliaceus. The mean age at disease onset for pemphigus vulgaris patients was 39.2 ±13.3 years with male to female ratio of 1:1.45. In the pemphigus foliaceus group the mean age at disease onset was 44.3 ± 12.9 years, male to female ratio of 3.5:1. In 82% pemphigus vulgaris patients the site of onset was oral mucosa. Mucocutaneous pemphigus was present in 92.5%, mucosal predominant in 5.5% and cutaneous predominant pemphigus in 1%. The mean PDAI score was 23.5 (range,1 to 75) in pemphigus vulgaris patients and 33.8 (range, 3 to 105) in pemphigus foliaceus patients.

Typical features of on histopathology was seen in 88.4% pemphigus vulgaris cases and 100% foliaceus cases. DIF was positive in 91.4% vulgaris patients and 100% foliaceus patients. The mean anti-Dsg1 and anti-Dsg 3 levels in patients with pemphigus vulgaris was 147.01 u/mL and 60.8 u/mL respectively. The anti-Dsg3 levels showed a positive correlation with the PDAI scores in pemphigus vulgaris patients ($p < 0.001$). The mean anti-Dsg1 in pemphigus foliaceus was 177.14 and it correlated positively with PDAI scores ($p < 0.01$).

HLA typing was done in 50 patients and compared to 50 controls. Significant association was seen with DRB1*14 and DQB1*05 with pemphigus vulgaris patients. HLA DRB1*14 was present in 94% cases and 36% controls ($p < 0.001$). HLA DQB1*05 was present in 94% cases and 48% controls ($p < 0.001$). The haplotype DRB1*14, DQB1*05 was present in 88% cases versus 28% controls ($p < 0.001$). Negative association was found with DRB1*15 (14% cases versus 32% controls, $p < 0.05$) and DQB1*06 (16% cases versus 38% controls, $p < 0.05$).

HLA DQB1*03 was associated with more severe disease.

Mean serum total IgG4 in pemphigus vulgaris patients was 1114.4 ±576 mg/L. Serum IgG4 levels had a positive correlation with anti-Dsg3 antibody levels ($p < 0.01$). There was a positive correlation between PDAI and IgG4 levels but did not reach significance levels.

Mean serum total IgG4 in pemphigus foliaceus patients was 1471.14 ± 1644.3 mg/L. It did not have a significant correlation with PDAI scores.

**Conclusion:** Pemphigus vulgaris is the most common type of pemphigus seen in India. The average age at onset of disease is similar to that reported from other Indian studies, and is lower than that reported from other parts of the world. Slight female predominance is seen in pemphigus vulgaris. HLA DRB1*14 and DQB1*05 alleles and the haplotype DRB1*14, DQB1*05 are strongly associated with pemphigus vulgaris. DQB1*03 is associated with more severe disease. Serum Dsg-3 levels correlated positively with PDAI scores in pemphigus vulgaris while anti-Dsg1 levels correlated with PDAI scores of pemphigus foliaceus patients. There was a weak association between serum total IgG4 levels and disease severity scores in pemphigus vulgaris but the same was not demonstrated in pemphigus foliaceus.

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# ANNEXURE 1

# DATA ENTRY PROFORMA

STUDY OF HLA TYPES AND SERUM IMMUNOGLOBULIN G4 IN PEMPHIGUS

DATE : STUDY NO: HOSPITAL NO:

NAME : AGE : SEX: M=1, F=2

ADDRESS :

FIRST VISIT/ REVIEW

Active disease / remission

DURATION OF ILLNESS: _____

SITE OF ONSET OF LESIONS : 1= MUCOSA / 2= SKIN/ 3= SCALP/ 4=NAILS

SYMPTOMS : 1= PAIN / 2= PRURITUS/ 3= BURNING

PHOTOSENSITVITY : 1=PRESENT / 2=ABSENT

DRUGS INDUCING / AGGRAVATING PEMPHIGUS : PRESENT / ABSENT

DETAILS ……

TREATMENT RECEIVED :

1= STEROIDS : ORAL / INJECTABLE

MAXIMUM DOSE OF STEROIDS (…..mg/kg) :

DURATION ……

2 = STEROID SPARING DRUGS ( WITH DOSE AND DURATION)

| DRUG | DOSE | DURATION | RESPONSE/ COMMENT |
|---|---|---|---|
| AZATHIOPRINE | | | |
| CYCLOPHOSPHAMIDE | | | |
| METHOTREXATE | | | |
| MYCOPHENOLATE | | | |

| | | | |
|---|---|---|---|
| MOFETIL | | | |
| IVIG | | | |
| RITUXIMAB | | | |

Currently on…

Stopped drugs when / how long back

ASSOCIATED COMORBIDITIES : present / absent

If Present-

THYMOMA/ MYASTHENIA GRAVIS/

DIABETES MELLITUS/ HYPERTENSION / CORONARY ARTERY DISEASE / METABOLIC SYNDROME /THYROID DYSFUNCTION

OTHERS-SPECIFY-

FAMILY HISTORY OF PEMPHIGUS : 1= PRESENT/ 2=ABSENT

(SIBLING/PARENT/CHILDREN)

<u>EXAMINATION</u>

SKIN ::

SITES INVOLVED - FACE/ TRUNK/ LIMBS/ FLEXURES/ GENITALS

LESIONS – BULLAE / EROSIONS/ HYPERPIGMENTED MACULES/

ACANTHOMA/ VEGETATING LESIONS

SECONDARY INFECTION : 1= PRESENT/ 2= ABSENT

MUCOUS MEMBRANES INVOLVED --

ORAL : LABIAL/ BUCCAL/ GINGIVAL/ TONGUE/ FLOOR OF MOUTH / PHARYNX

NASAL ; INVOLED / NOT INVOLVED

CONJUNCTIVA: 1=INVOLVED, 1.A=UNILATERAL, 1.B =BILATERAL

2=NOT INVOLED

GENITAL MUCOSA: 1=INVOLVED / 2=NON INVOLVED

SCALP ; 1= INVOLVED / 2=NOT INVOLVED

NAILS : PERIONYCHAL LESIONS 1=PRESENT/2= ABSENT

NIKOLSKY'S SIGN : DIRECT:: 1=POSITIVE / 2=NEGATIVE

PERILESIONAL:: 1=POSITIVE/ 2=NEGATIVE

BULLA SPREAD SIGN : 1=POSITIVE / 2=NEGATIVE

<u>INVESTIGATIONS</u>

VITAMIN D LEVELS:

DESMOGLEIN 1 :

DESMOGLEIN 3 :

SERUM IgG4 :

TZANCK :

BIOPSY :

DIF :

HLA TYPE : DR…. DQ…..

# ANNEXURE 2

## PEMPHIGUS DISEASE ACTIVITY INDEX

PDAI –SKIN :

ACTIVITY SCORE 0= absent,

1=1-3 lesions, upto 1>cm none>6cm

2=2-3 lesons, at least 2>2cm, none >6cm

3= >3 lesions, none >6cm

5 = >3lesions, and/or at least one >6cm

10 = >3lesions, and/or at least one >16cm/ entire area.

| Anatomical location | Activity score |
|---|---|
| Ears | |
| Nose | |
| Rest of face | |
| Neck | |
| Chest | |
| Abdomen | |
| Back/buttocks | |
| Arms | |
| Hands | |
| Legs | |
| Feet | |
| Genitals | |
| Total skin ( /120) | |

PDAI- SCALP EROSION/ BLISTER OR NEW ERYTHEMA

0= absent

1= one quadrant

2= two quadrants

3= three quadrants

4=whole scalp

10= at least one lesion >6cm

DAMAGE SCORE – PIH, ERYTHEMA FROM RESOLVING LESION

0=Absent

1= Present.

| Anatomical location | Damage score |
|---|---|
| Ears | |
| Nose | |
| Rest of face | |
| Neck | |
| Chest | |
| Abdomen | |
| Back/buttocks | |
| Arms | |
| Hands | |
| Legs | |
| Feet | |
| Genitals | |
| Scalp | |
| Total skin (  /120) | |

PDAI- MUCOUS MEMBRANES - erosions/ blisters.

0=absent 1= 1 lesion 2= 2-3 lesions

5= >3 lesions or 2 lesions >2cm 10= Entire area

| Anatomical location | Activity score |
|---|---|
| Eyes | |
| Nose | |
| Buccal mucosa | |
| Hard palate | |
| Soft palate | |
| Upper gingiva | |
| Lower gingiva | |
| Tongue | |
| Floor of mouth | |
| Labial mucosa | |
| Posterior pharynx | |
| Anogenital | |
| Total ( /120) | |

| | | |
|---|---|---|
| 1 | Skin activity | |
| 2 | Damage score | |
| 3 | Scalp score | |
| 4 | Mucosal score | |
| 5 | Total PDAI (1+2+3+4) ( /263) | |

## ANNEXURE 3

## INFORMED CONSENT FORMS

**PATIENT INFORMATION SHEET**

TITLE : STUDY OF HLA DR AND DQ TYPES IN PATIENTS WITH PEMPHIGUS COMPARED TO THAT IN HEALTHY SUBJECTS.

We are doing a study on patients with pemphigus. Pemphigus is the name given to a group of diseases that cause blisters on the skin and erosions in the mouth

1.. Patients with this disease have been found to have certain types of Human Leukocyte Antigen (HLA). HLA is the name given to genes that are related to immune system function (the system that fights against infections in our body). There are many types of HLA of which HLA DR and DQ types are associated with pemphigus. These HLAs have been reported in studies done in other countries.

We are doing this study to find out if these types of HLA are more common in Indian patients with pemphigus.

To find out the HLA type we will be taking 9mL of blood sample from your arm using a needle and syringe. For this study purpose blood sample from you will be taken only once.The test might provide knowledge about the reasons for developing this disease.

2. . The severity of the disease can be assessed by certain laboratory parameters which includes the immunoglobulin G4 levels in the blood.

IgG4 is a protein that is produced normally by the body in low levels, it can be found in highly increased amounts during disease process. Higher levels of the antibody suggests a more active disease. To find out the serum IgG4 levels we will be taking 3mL of blood sample from your arm using a needle and syringe. The test is done in all patients with pemphigus alongwith other routine blood tests. It is not associated with any significant adverse events. Based on the IgG4 levels in your blood the dose and the duration of your therapy might change. We are inviting all patients with pemphigus to participate in our study.

The information that we collect from you in this research project will be kept safe with us and no-one other than the reseachers will be able to see it. If this study is published your identity will not be revealed. Your participation in the study is voluntary and you can choose not to participate in the study at any time. Your treatment will continue without any change if you choose not to participate in the study.

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact us at the following:

Dr.Anuradha Priyadarshini
Postgraduate registrar, Dept of Dermatology, Christian Medical College, Vellore. Phone - 0416-2283527. Email - anupriyadarshini@gmail.com

## PARTICIPANT INFORMATION SHEET (FOR CONTROLS)

TITLE: STUDY OF HLA DR AND DQ TYPES IN PATIENTS WITH PEMPHIGUS COMPARED TO THAT IN HEALTHY SUBJECTS

We are conducting a study on patients with pemphigus. Pemphigus is a skin disease that is associated with blisters on the skin and ulcers in the mouth. The exact cause of this disease is not known but it has been seen that patients with pemphigus have certain specific types of HLA. HLA (Human Leukocyte Antigen) is the name given to genes that are related to immune system function (the system that fights against infections in our body). There are many types of HLA of which HLA DR and DQ types are associated with pemphigus. These HLAs have been reported in studies done in other countries.

We are doing this study to find out if these types of HLA are more common in Indian patients with pemphigus.

To compare the frequency of these HLA types with normal people we wish to use the results of the HLA tests that have been done on you for the purpose of HLA matching while you were donating the organ for your relative.

The use of this data will not affect the treatment of your relative.

The information that we collect from you in this research project will be kept safe with us and no-one other than the reseachers will be able to see it. If this study is published your identity will not be revealed.

You will not gain anything by participating in this study. But the study might provide knowledge about the reasons for developing this disease.

Your participation in the study is voluntary and you can choose not to participate in the study at any time. I am mailing you the consent letter. Kindly sign it and mail it back.

If you have any questions you may ask them now or later. If you wish to ask questions later, you may contact us at the following:

Dr.AnuradhaPriyadarshini

Postgraduate registrar, Dept of Dermatology, Venereology and Leprosy,

Christian Medical College, Vellore 632004

Phone - 0416-2283527.

Email - anupriyadarshini@gmail.com

## INFORMED CONSENT FORM

**Study Title: : To study the HLA DR and DQ types in patients with pemphigus as compared to that in healthy subjects**

**Study Number: _____**

**Subject's Name: _____**

**Date of Birth / Age: _____**

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions.

(ii) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

(iii) I understand that the Sponsor of the clinical trial, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published.

(iv) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).

(v) I agree to take part in the above study.

Signature (or Thumb impression) of the Subject/Legally Acceptable Representative

Signature: _____ Date: _____/_____/_____

Signatory's Name: _____

Signature of the Investigator: _____ Date: _____/_____/_____

Study Investigator's Name: _____

Signature of the Witness: _____ Date: _____/_____/_____

Name & Address of the Witness: _____

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நோயாளிகள் தகவல்

தலைப்பு: பெம்பிகஸ் நோயாளிகள் மற்றும் நோயற்றவர்களின் ஹெச்.எல்.ஏ டி.ஆர் மற்றும் டி.க்யூ பற்றிய ஆய்வு.

பெம்பிகஸ் நோயைப் பற்றியதோர் ஆய்வை மேற்கொள்ள உள்ளோம். பெம்பிகஸ் என்பது தோல் கொப்புளங்கள் மற்றும் வாய்ப்புண்களுடன் கூடியதோர் நோய். இந்த நோயுடையவர்கள் சில குறிப்பிட்ட விதமான ஹெச்.எல்.ஏ மரபு - அணுக்கள் உடையதாக அறியப்பட்டுள்ளது. நோய் எதிர்ப்பு சம்பந்தப்பட்ட மரபு - அணுக்களை ஹெச்.எல்.ஏ என்று குறிப்பிடுகிறோம். இவற்றில் பல விதங்கள் உண்டு. அவற்றில் ஹெச்.எல்.ஏ டி.ஆர் மற்றும் ஹெச்.எல்.ஏ டி.க்யூ பெம்பிகஸ் நோயுடன் தொடர்புள்ளதாக தெரியவந்துள்ளது.

இதை பற்றியதான ஆய்வுகள் வெளிநாடுகளில் மேற்கொள்ளப் - பட்டுள்ளன. இந்தியாவில் உள்ள பெம்பிகஸ் நோயாளிகளில் இவ்வித ஹெச்.எல்.ஏ விதங்கள் உள்ளனவா என்பதைப் பற்றிய ஆய்வை மேற்கொள்ள உள்ளோம். இந்த ஆய்வில் பங்கேற்க அனைத்து பெம்பிகஸ் நோயாளிகளையும் வேண்டிக் கொள்கிறோம்.

ஹெச்.எல்.ஏ.வை நிர்ணயிக்க தங்களிடமிருந்து சுமார் 5.மி.லி. இரத்தம் பரிசோதனைக்காக எடுக்கப்படும். இரத்தம் எடுக்கப்படும் இடத்தில் இருந்து சிறுதளவு இரத்தக்கசிவு ஏற்படலாம். இந்த ஆய்வுக்காக ஒருமுறை மட்டுமே தங்களிடமிருந்து இரத்தம் எடுக்கப்படும்.

இந்த ஆய்வின் போது தங்களைப் பற்றியதான விவரங்கள் தக்க முறையில் பாதுகாக்கப்படும். ஆய்வு சம்பந்தப்பட்டோர் தவிர்த்து மற்று ஒருவரும் உங்களைப் பற்றிய விவரங்களை அறிய இயலாது. இந்த ஆய்வின் முடிவுகள் வெளியிடப்பட்டால் அதில் தங்களை குறிக்கும் விவரங்கள் வெளியிடப்படாது.

இந்த ஆய்வில் பங்கேற்பதின் விளைவாக நாங்களுக்கு நேரடியாக ~~எதுவும்~~ பயனில்லை எனினும் இந்த நோயின் காரணத்தை பொதுவாக அறிய உதவும்.

இந்த ஆய்வில் பங்கேற்பது நாங்களின் விருப்பத்தை சார்ந்தது. இந்த ஆய்வில் நீங்கள் பங்கேற்காவிடினும், அது நீங்கள் இந்த மருத்துவமனையில் எடுக்கும் எந்த சிகிச்சையிலும் மாறுதலை உருவாக்காது.

இந்த ஆய்வைப் பற்றிய தகவல்களை அறிய விரும்பினால், கீழ்கண்ட மருத்துவரை அணுகலாம்.

டாக்டர். அனுராதா ப்ரியதர்சினி

தொலைபேசி எண் - 0416 - 228 3527

# தெரிவுத்தாள் படிவம்

மருத்துவ மாதிரியில் பங்குகொள்வதற்கான தெரிவுத்தாள் படிவம்

தலைப்பு:- பெம்பிகஸ் நோயாளிகள் மற்றும் நோயற்றவர்களின் பெரிஃபெரல் எஸ். ருடி. ஆர் மற்றும் டி.சீயு பற்றிய ஆய்வு.

பாடத்தின் எண்:

பிறந்த தேதி/ வயது :

நான் _____, ஆண்/பெண் of _____

கீழ்த்து: தேதியன்று கீழ்த்த படிவத்தையும் மேற்கண்ட உரிப்பின் நோக்கத்தையும் நன்கு அறிந்துக் கொண்டேன்.

இது சம்மந்தமாக எனது பங்கு தற்காலிகமானது எனவும் எனது மருத்துவ சிகிட்சை மற்றும் உரிமை பாதிக்காதவாறு எந்தவித காரணமும் இன்றி விலகிக் கொள்ளலாம் என்று புரிந்து கொண்டேன்.

கீழ்த்த படிப்பு சம்மந்தமாக பொறுப்பில் உள்ளவர்கள் சட்டபூர்வமான முறையை சார்ந்தவர்கள், ஏதேனும் பரிசோதியவர்கள் ஆகியோர்கள் படிப்பு சம்மந்தமாகவோ அராய்ச்சி சம்மந்தமாகவோ என்னுடைய மருத்துவ பதிவேடுகளை கையான நான் கீழ்த்த படிப்பில் இருந்து விலகிக் கொண்டாலும் அனுமதி தேவையில்லை என்பதை சம்மதிக்கிறேன்.

நோயாளி / சட்டபூர்வ அனுமதி அளிக்கப்பட்டவர்
கையொப்பம் / கை ரேகை:

தேதி: ___/___/___

கையெழுத்திடுபவரின் பெயர்: _____

பரிசோதிப்பவரின் கையொப்பம்:- _____

தேதி: ___/___/___

பரிசோதிப்பவரின் பெயர் : _____

சாட்சியாளரின் கையொப்பம்:- _____

தேதி:- ___/___/___

சாட்சியாளரின் பெயர்:- _____

# সম্মতি পত্র

শিরোনাম - স্টাডী অফ এচ.এল.এ ক্লাস এবং ডিজিজ টাইপ ইন পেশেন্টস উইথ পেমফিগাস বোলমেয়েআর্ড ইন হেলদী আবছেক্ট

আমাদের অধ্যয়ন পেমফিগাস নিয়ে. পেমফিগাস এই রোগটাতে ভুল ফোসকা হয় চামড়া ও মুখের ভিতোরে. এই রুগীদের মধ্য কিছু নির্দিষ্ট HLA ধরন দেখা দিয়েছে. HLA মানে হচ্ছে জিন গুলো শরীরের প্রতিকার আক্তিতে আন্তাক্তো করে. অনেক ধরনের HLA আছে তার মধ্যে DR এবং DQ ধরন পেমফিগাসের সঙ্গে জরিত।

আমাদের অধ্যয়নের দারা কোন ধরনটা ভারতিয় দের মধ্য আছে তা বোঝা জাবে.

আমরা আব পেমফিগাস রুগীদের এই অধ্যেয়নে আমন্ত্রণ জানাই.

এই অধ্যেয়নের জন্য 5ml রক্ত (রক্ত) নেয়া হবে নিডেল এবং সিরিঞ্জের দারা. এইটি খালি এক বার করা হবে.

এই অধ্যেয়নের তথ্য পুরোপুরী গোপনিয়.

এই অধ্যেয়নের দারা আপনার কোনা লাভ হবে না, কিন্তু এটা পরোবর্তি চিকিৎসা ও অন্য রুগীদের কাছে লাগবে

আপনার জোগদান আপনার উপোর. আপনার চিকিৎসাতে কোনো বাধা আসবেনা.

আপনার কোনো প্রশ্ন থাকলে এখন বা পরে বলতে পারেন.

প্রশ্ন থাকলে নিচের ঠিকানাতে যোগাযোগ করুন.

ডা: অনুরাধা প্রিয়াদর্শিনী
পি. জি রেসিডেন্ট, সি.এম.সি ভেলোর ৬৩২০০৪ (632004)
ফোন - ০৪১৬-২২৮৩৫২৭ (২২৪৩৫২৭) (0416)

শিরোনাম - স্টাডী অফ এচ.এল.এ টীকার এবং ডিফিকাল্ট টাইপ ইন মেক্সানটিস অইত মেমফিগস রোগমযেআর্ত ইন হেলদী আবছে।

স্টাডি নং:-

রোগীর আক্ষর -

রোগীর নাম -

জন্মতারিখ -

① আমি ক্ষুদ্রভাষ্ট উপরে তথ্য পড়ে বুঝেছি এবং আমি বুঝেছি যে আমার প্রশ্ন করার সুযোগ আছে।

② আমি বুঝেছি যে আমার অংশগ্রহন আমার ইচ্ছা অনুযায়ী এবং যেকোনো সময় আমি আমার নাম তুলে নিতে পারি কোনো কারন দর্শানো ছাড়াই। এর জন্য আমার চিকিৎসার কোনো ক্ষতি হবে না।

③ আমি বুঝেছি যে রেস্পন্সিবল বোর্ড থেকে আমার অনুমতি নেওয়ার প্রয়োজন হবে না, আমার রেকর্ড (চিকিৎসা) দেখার জন্য, আমি যদি নাম তুলে নিই তবুও দেখতে পারে এবং আমার তথ্য অন্য কাউকে জানানো হবেনা।

④ আমি এই গবেষনা থেকে যে তথ্য পাওয়া যাবে তা অন্য গবেষনা তে ব্যবহার করার জন্য অনুমতি দিচ্ছি।

⑤ আমি এই গবেষনা তে অংশ গ্রহন করতে ইচ্ছুক।

রোগীর আক্ষর - ____ তারিখ :-

পরিবারের আক্ষর: ____ তারিখ:-

সাক্ষীর আক্ষর- ____ তারিখ:-

కైటిల్ :- (Pemphigus) పెంఫిగస్ తో ఉన్న రోగులలో ఉండే HLADR మరియు DR అంశాల గురించి తెలుసు కొని మన యొక్క అధ్యయన subject తో పోల్చుకోవడం.

రోగి యొక్క సమాచారం (వివరాలు) సూచిక :—

మేము పెంఫిగస్ (Pemphigus) ఉన్న బాధితుల తో ఉన్న కొంతమంది రోగులను పరిశోధన (study) చేస్తున్నాము. పెంఫిగస్ (Pemphigus) అనునది కొన్ని రోగముల సమూహాయం. ఈ బాధిత కారకాలు చర్మము మీద బొబ్బలు (blisters) రావడం, నోటి పుండు మొదలగునవి, ఈ రోగంతో ఉన్న వారిలో కొన్ని రక్తాలు (HLA) హ్యూమన్ ల్యూకోసైట్ యాంటిజన్ (Human Leukocyte Antigen) అను పేరు గల జీన్స్ (Genes) తో సంబంధం గల వ్యాధి నిరోధక వ్యవస్థ పనుల మార్పులు మానవ శరీరంలో కనుగొనబడినది. (Immune system Function). చాలా రకాల హ్యూమన్ ల్యూకోసైట్ యాంటిజన్ (HLA) యొక్క HLA DR (హ్యూమన్ ల్యూకోసైట్ యాంటిజన్ - డి.ఆర్) మరియు DR (డి.క్యూ) యొక్క రక్తాలు (pemphigus) పెంఫిగస్ అను బాధితో సంబంధం కలదు. ఈ HLAs యొక్క సమాచారాన్ని వేరు వేరు దేశాల కు వివరించడం జరిగింది.

భారత దేశపు రోగులలో పెంఫిగస్ తో బాధపడే రోగులలో ఎంత మంది ఈ HLA అను రకం బొబ్బలు బాధపడుతున్నారు అని మేము సమాచారాన్ని సేకరిస్తున్నాము.

మేము చేసే ఈ పరిశోధనలో పెంఫిగస్ తో బాధపడే ప్రతి రోగి పాల్గొనవచ్చును - మరియు పాల్గొనుటకు ఆర్థికం లేదు అని మేము పలుకుతున్నాము.

HLA అను రకాన్ని కనుగొనుటకు మేము ప్రతి రోగి నుండి 5 టీస్పూన్ల (5 ml) ల రక్తాన్ని మీ యొక్క చేతి నుండి సేకరించి మరియు మా ల్యాబ్ లో టెస్ట్ చేస్తాము. ఒకే ఒక్కసారి మీ నుండి రక్తం తీసుకొన బడును. రక్తం తీసుకున్న చోట కొద్దిగా చిన్న నొప్పి మరియు చిన్నగా రక్తస్రావం ఉండును.

ఈ పరీక్ష చేయించు కోవడం వలన మీకు ఎటువంటి హాని తలపెట్టకుండా ఉండదు. కాకపోతే ఈ వ్యాధి గురించి ఎవరూ గనను పెంచుకోవచ్చు

ఈ పరీక్ష లో పాల్గొన్న వారి వివరాలు గోప్యంగా ఉంచబడును.
మీయొక్క పరీక్ష ఫలితాలను విచారణ చేసే వారికి మాత్రమే
చూస్తారు. వేరే ఎవరికీ కూడా ఇవ్వరు లేదా ఎవరికీ కూడా
గోప్యంగా ఉంచబడును.

ఈ HLA ను విచారణ పరీక్షలో మీకు ఇష్టం ఉంటేనే
పాల్గొనవచ్చును. ఇష్టం లేనిచో విరమించుకోవచ్చును.
మీరు పాల్గొనకపోయినా మీకు ఉన్న వ్యాధికి చికిత్స
continue చేయబడును. ఇందులో ఎటువంటి మార్పు
లేదు.

మీకు ఏమైన సందేహాలు ఉంటే పెండింగ్ ఉంచకుండా
లేదా ఈ పరీక్ష జరిగిన తరువాత ఉన్నా ఎప్పుడు
సందేహాలను తీర్చుకోవచ్చును.

ధన్యవాదములు.


డా. అనురాధ ప్రియదర్శిని,
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ప్రొటోకాల్ :- (Pemphigus) పెంఫిగస్ తో ఉన్న రోగులలో ~~ఉండే~~ ~~HLA~~ HLADR మరియు ఇతర తరగతుల జన్యువులు తెలుసుకొని మన యొక్క లిటరేచర్ study తో పోల్చుకోవడం.

Study Number:

Date of Birth / Age: _____

పుట్టిన తేదీ / వయస్సు: _____

నేను _____

_____, కొడుకు/కూతురు (son/daughter) కుమార్తె _____

(1). నా కవ్వబడిన సమాచారము నేను చదివి గ్రహించిన దేమనగా, అది రూఢియని నిర్ధారించు చున్నాను. ఇలాంటి ఈ సమాచారము నందు ప్రశ్నలడుగుటకు ఇవకాశము కలిగినది.

2. ఈ విషయము లో నా ప్రవేయము పూర్తిగా స్వేచ్ఛా ధ్రువీకరణ మైనది అయినా, ఏ సమయంలో నైనను కారణము తెలుపక యే విరమించు కొను స్వేచ్ఛ కలదు అని నేను గ్రహించితిని.

3. నేను తరగతి సంబంధమైన పరిశోధన సంబంధ విషయాంలో పరిమితి కొన్న బృందికి, ఆ కార్యక్రమాలు పరిశీలించేందుకు రోగ పరీక్షలకు సంబంధించిన సాధికారులు సాధికారులు తరపున పనిచేసే ఇతరులు, నైతిక విలువలు పరిక్షించే కమిటి సియంత్రణ ఉద్యోగులకు నా ఉమతి ఇవసరము లేదని గ్రహించితిని. నా ఉనికి ఎ సమాచారము బాహ్యంగా బయట్టపరుచు చేయబడదని, మూడవ వ్యక్తికి తెలియ చేయు బడుటగాని, ప్రచురించుటగాని, జరగదు అని నేను గ్రహించి ఈ పరిశోధనలో ప్రవేశాన్ని ~~ఇవకరిం~~ అంగీకరించితిని.

4. ఈ విషయము బాహ్యంగా వచ్చు పరిశోధనా ఫలితములను, శాస్త్ర సంబంధమైన విషయాలలో ఉపయోగించు కొనుటకు ఉద్యోగులు లేవని — అంగీకరించు చున్నాము.

5. ఈ విషయములోగా పొందొనుటకు నేను అంగీకరించు చున్నాను.

- సంతకము (లేదా) వేలిముద్ర - అంగీకారము తెలుపు ప్రతినిధి: _____
- పరిశోధకుని పేరు: _____ మరియు (date) తేదీ:
- విషయ శోధకుని పేరు: _____
- సాక్షి సంతకము: _____ మరియు తేదీ:
- సాక్షి పేరు: _____

# अध्ययन सूचना पत्र

शोध का शीर्षक : पेम्फीगस (PEMPHIGUS) के मरीजो में एच. एल. ए डी. आर (HLA DR) एवं डीक्यू के प्रकार तथा स्वस्थ व्यक्तियों से उसकी तुलना

हम पेम्फीगस के मरीजो पर एक अनुसंधान कर रहे है। पेम्फीगस नामक रोग मे मनुष्य के शरीर पर फोडे एवं मुहँ मे छाले निकलते है। इस रोग से पीडित मरीजो मे विशेष प्रकार के एच. एल. ए (H.L.A) पाए जाते है। एच एल ए एक जीन (gene) समूह है जो मनुष्य के रोगक्षम (बिमारी से लडने अथवा बचाव की क्षमता) से सम्बंधित है।

एच एल ए विभिन्न प्रकार के होते है, जिनमे डी आर (DR) एवं डी क्यू (DQ) प्रकार के एच एल ए पेम्फीगस रोग से सम्बंधित है। इस विशय मे विदेश मे कई अनुसंधान किए गए है। इस अनुसंधान के द्वारा हम यह जानना चाहते कि क्या इन प्रकार के एच एल ए भारतीय पेम्फीगस मरीजो में अधिक संख्या में पाए जाते है।

हम पेम्फीस से पीडित सभी मरीजो को इस अनुसंधान में शामिल करना चाहते है।

इस अध्ययन के लिए हम आपके बाँह के नस (इग से 5 mL (पाँच मिली.) रक्त लेंगे। जहाँ से रक्त लिया जाएगा वहाँ से कुछ रक्त जाव होने की सम्भावना है।

इस अनुसंधान के लिए आपसे मात्र एक बार रक्त लिया जाएगा।

इस अध्ययन के द्वारा जो जानकारी प्राप्त होगी वह हमारे पास सुरक्षित रखी जाएगी। यदि यह अनुसंधान प्रकाशित होता है तो उसमे आपकी पहचान नहीं प्रकाशित होगी।

आपको इस अनुसंधान से कोई विशेष लाभ नहीं होगा परन्तु इस अध्ययन से इस रोग के उत्पत्ति के विषय मे जानकारी प्राप्त हो सकती है।

आपका इस अनुसंधान मे भाग लेने का निर्णय आपकी अपनी इच्छा से है। यदि आप इसमे भाग नहीं लेना का निर्णय करते है तो आपका इलाज निर्धारित प्रक्रिया के अनुसार बिना बदलाव के चलता रहेगा।

यदि आप इस विषय में कोई सवाल करना चाहे तो कृपया निम्न लिखित पते अथवा फोन नम्बर पर संपर्क करे।

डॉ. अनुराधा प्रियदर्शिनी

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# सहमति पत्र

शोध का शीर्षक : पेम्फीगस (PEMPHIGUS) के मरीजो मे HLA DR. एवं DQ. के प्रकार तथा स्वस्थ व्यक्तियों से उसकी तुलना

शोध क्रमांक : _____ नाम : _____

आयु : _____

1. मैंने उपरोक्त शोध अध्ययन के सूचना पत्र को पढ़ एवं समझ लिया है। मुझे उससे सम्बंधित प्रश्न पूछने का अवसर मिला है।

2. मैं जानता हूँ कि शोध में भागीदारी संपूर्ण रूप से मेरी मर्ज़ी और सहमति से है और मैं किसी भी समय इसमें भाग लेने से इनकार कर सकता हूँ और इससे मेरे चिकित्सा अथवा कानूनी अधिकार पर प्रभाव नहीं होगा।

3. मैं जानता हूँ कि शोध अध्ययन के स्पोंसर एवं उससे जुड़े लोगो को मेरा स्वास्थ्य चिकित्सा रेकॉर्ड इस शोध अध्ययन और आगे अन्य अनुसन्धान मे शामिल करने हेतु मेरी अनुमति की जरूरत नहीं होगी। मैं जानता हूँ कि मेरी पहचान और अन्य जानकारी किसी भी रूप से किसी तीसरे पक्ष को नहीं बताई जाएगी / या प्रकाशित नहीं की जाएगी।

4. मैं इस शोध अध्ययन के किसी भी जानकारी या परिणाम के वैज्ञानिक उद्देश्य हेतु उपयोग में बाधक नहीं बनूँगा

5. मैं इस शोध अध्ययन मे भाग लेने के लिए सहमति देता हूँ।

मरीज़ / भागीदार / कानूनी प्रतिनिधि के
हस्ताक्षर / अंगूठे का निशान : _____

हस्ताक्षर करने वाले का नाम _____ दिनांक : _____

शोध कर्ता के हस्ताक्षर : _____ दिनांक : _____

शोध कर्ता का नाम : _____

गवाह के हस्ताक्षर : _____

गवाह का नाम : _____ दिनांक

# ANNEXURE 4

## HLA DR AND DQ TYPING BY PCR –SSOP METHOD

## PRINCIPLE:

PCR amplification of DNA is used as the means to enrich selected DNA region. During the initial cycles of amplification step, double stranded DNAs are generated. On the exhaustion of the limiting primer, the remaining primer uses the double stranded product as template for generation of single-stranded DNA. SSO typing procedure is based on the hybridization of labeled single stranded PCR product to SSO probes. Each of the different probes is designed so that each probe preferentially hybridizes to a complementary region that may or may not be present in the amplified DNA.

Different probes are attached to up to 100 different colored Luminex Microspheres, designed for use with the Luminex instrument. The Luminex instrument is able distinguish each microsphere and quantify the relative amounts of labeled PCR product hybridizing to each Luminex microsphere. Therefore, the relative signal obtained with the SSO probes can be used to assign the probes as having positive or negative reactivity with the amplified DNA sample. This in turn provides the information needed to determine the HLA phenotype of the sample.

The beads are analyzed using the Luminex Flow Analyzer and the Luminex IS software. The Quick type for Life match software is then used to evaluate specimens for reactivity and print the reports.

**SAMPLING AND STORAGE**:

DNA: The genomic DNA is extracted from the blood using Salting-out technique (refer DNA extraction protocol). The concentration of the DNA is adjusted to 150 ŋg /µl using the formula 15 X 200 / the total concentration of the DNA.

We need 150 ŋg /µl of DNA i.e. 10µl of diluted DNA for each test well.

Storage: The micro tube containing the assayed DNA is stored at –80 °C freezers to maintain the integrity of the DNA.

**MATERIALS**:

Luminex Flow Analyzer with Luminex IS and Quicktype for Lifematch software

ABI Thermal cyclers

Vortex

Water baths with 37 °C and 56 °C temperature.

PCR tubes and caps

Thermal cycler (PCR) 96 well plates

Micro seal Film

Barrier filter tips

Pipettes → Variable pipettes – (10-1000µl), (0-10µl), (5-50µl), (20-200µl).

Multi-channel pipettes

**REAGENTS:**

Recombinant Taq Polymerase

Nuclease-Free Water

**LIFECODE HLA-DRB1 Typing test kit consist of**

1. Lifecode HLA DRB Mastermix
2. Lifecode HLA DRB Probemix

**LIFECODE HLA-DQB Typing test kit consist of**

3. Lifecode HLA DQB Mastermix
4. Lifecode HLA DQB Probemix

Lifecode R-Phycoerythrin Conjugated Streptavidin (SA-PE)

**PROCEDURE**

Preparation OfMastermix:

1. The mastermix that is stored at 2 – 8 °C, should be taken out from storage just prior to use.
2. Take out the mastermix from the corresponding kit box. Place the mastermix in 37 °C water bath for 10 minutes (to warm up).
3. Requirement for each test is,

   24.5 µl of water/well

   15 µl of the appropriate mastermix/well

   0.5 µl of Taq/well.

While preparing the mix, the required volume of the above reagents per panel is calculated as per the Number of tests + Negative control + 1 well extra (to account for pipetting error).

**Amplification:**

1. Take an 8 -cap strip (*Micro amp; Applied Biosystems*)
2. Cut to separate the number of wells required.
3. Label the wells visibly with the DNA sample number on the sides, and the panel ID.

4. Place the wells in the rack.
5. Add 10µl of diluted DNA of the patient horizontally.
6. Add 40 µl of the corresponding mastermix in the wells vertically.
7. Add cover with seal.
8. Check tubes to ensure proper pipetting. If necessary, tap tubes to make sure all reagents are at the bottom of the tube.
9. Cap tubes tightly to prevent evaporation during PCR run.
10. The final volume must be 50µl. Place the tubes in the thermal cycler.
11. Press the programme for Luminex amplification, which is as follows.

| |
|---|
| 95° C for 5 min |
| Number of cycles: 1 |
| 95° C for 30 sec |
| 60° C for 45 sec |
| 72° C for 45 sec |
| Number of cycles: 8 |
| 95° C for 30 sec |
| 63° C for 45 sec |
| 72° C for 45 sec |
| Number of cycles: 32 |
| 72° C for 15 min |

**Hybridisation :**

The probe mix is stored in the fridge at 2 – 4 °C for 10 minutes prior to use, take out the probe mixers from the respective box and keep in the water bath at 56°C.

Meanwhile, label the coaster plate in a fashion similar to placement of the wells in the rack previously i.e. samples assigned to rows and panels to columns. (See diagram)

1. Vortex and add 15 μl of probe mix to the wells
2. Then add 5 μl of the amplified product to the test wells.
3. Seal with the coaster seal provided.
4. Place them in the Thermal cycler and start the hybridization programme, which is as follows.

| |
|---|
| 95° C for 5 min |
| 47° C for 30 min |
| 56° C for 10 min |
| 56° C HOLD |

5. When 10 minutes is to over, prepare SAPE.

**SAPE Preparation**:

1. The SAPE is kept in the fridge at 2 – 4 °C. It must be vortexed before use. The solution is prepared as follows
2. Dilution solution provided in the kit –170 μl/well.
3. SAPE --- 0.85 μl/well.
4. Calculation of the quantity required per panel eg. ( 3 Tests + 1 NC + 1 extra well)
5. After preparation of the solution, it must be kept in the dark till use.
6. When the run is over, do not remove the plate from the Thermal cycler. Immediately, add 170 ul of the prepared SAPE solution to each well while the coaster plate is at 56° on the Thermal cycler.
7. Then, without delay, take the coaster plate and put it on the metal plate provided in Luminex XYZ platform. Click ‘Start. Plate’ on the software to start the run.

**Interpretation of the results**:

Analysis is comprised of the following steps:

1. Verify that the number of events for each SSO in each sample is at least 6. This information is found in the Data type : count section of the CSV file.
2. Determine that the values for the consensus probes for each sample are above their minimum Median Fluoroscent Intensity or MFI.

   The minimum thresholds are lot specific and can be found in the threshold table.
3. Subtract the Background Control value for each probe from the sample values producing the background corrected data set. Background values are average MFI values for each bead to compensate for background noise due to bead variation.
4. For each sample, divide the background-corrected data for each probe by the background-corrected value for the corresponding consensus probe producing the normalized data set.

MFI (probe) – MFI (control blank for probe)

MFI (consensus) – MFI (control blank for consensus)

1. For each probe, record the normalized value on the Threshold Table Worksheet.
2. Once all values have been assigned, the probe hit pattern (i.e., the combination of all positive and negative assignments for a given sample) can be compared with the Probe Hit Table provided.

References: www.gen-probe.com/lifecodes/documents

Product insert – Lifecodes HLA-SSO typing kits for use with Luminex

# ANNEXURE *5*
## Serum Total Ig4 Level Estimation

MININEPHHuman IgG4 Kit, product code ZK009.L.R, the Binding Site.

Principle : The determination of soluble antigen concentration by nephelometric method involves a reaction with antibody bound to latex particle to form insoluble complexes. When light is passed through the suspension formed a portion of the light is scattered and detected by a photodiode. The amount of light scattered is directly roportional to the specific protein concentration in the test sample. Concentrations are automatically calculated by reference to a calibration curve stored within this instrument.

Sample: Blood sample collected by venipuncture is allowed to clot naturally and serum separated as soon as possible to prevent hemolysis. Sera is stored at 2-8°C for upto 2 days or aliquot and freeze at -20°C or below. Dilutions are prespared on the say of assay.

**Reagents ;**

1. Human IgG4 reagent – monospecific antiserum coated onto polystyrene microparticles, supplied in lyophilized form.
2. Minineph IgG4 swipe card - encoded with details of reaction curve specific to respective lot of reagent
3. Buffer
4. High and low controls of pooled serum

**Procedure :**Summary of reagent volumes added to the cuvette ;

| Reagent | Volume added |
|---|---|
| Sample (1/121 dilution) | 20µL |
| Buffer | 400µL |
| IgG4 reagent | 40µL |

The analyzer is switched on and the chemistry number (IgG4 =9) is entered.

The chemistry card is swiped and the lot number is checked.

Dilutions of controls and samples using the sample diluent is prepared, recommended dilution of 1/121.

Cuvettes are prepared with 20µL of sample to which 400µL of buffer and 40µL of reagent are added, it is stirred and placed in the cuvette chamber.

Once the buffer and reagent are added and stirred, it is detected by the device and the assay begins. The results are printed out after 165seconds.

If the result is higher than the measuring range the sample is re-assayed at higher dilutions. If it is lower than the measuring range the sample is re-assayed at lower dilutions.

**Interpretation of results :**

Results are calculated by the instrument and displayed in mg/L.

Sample concentrations up to at least 3350mg/L will not result in antigen excess. Higher concentrations may give misleading results and should be re-assayed with dilutions.

**Limitations :**Nephelometric assays are not suitable for high lipemic or hemolysed samples or samples containing high levels of circulating immune complexes due to unpredictable degree of non-specific scatter these sample types may generate.

**Expected results :**

The IgG4 results obtained with normal adult donor sera is mean = 426m/L, median = 423mg/L, $95^{th}$ percentile range = 62-1127 mg/L

# ANNEXURE 6

# KEY TO MASTERCHART

Sno- study number

Pv- Pemphigus vulgaris, pf-pemphigus foliaceus, cc-controls

Hosp- hospital number

Age- age of patient, in years

Gend- gender of the patient,

1=male, 2=female

Place-place of residence,

1=TN Tamil nadu, 2=AP AndhraPradesh 3=kerala 4=westbengal 5=bihar

6=jharkhand 7=chattisgarh 8=others (

Visit- first or review visit,

1=first visit 2=review visit

Duran- duration of diesase in years

Onst-site of onset of lesions,

1=oral, 2=skin, 3=scalp

Symp- presenting symptom at disease onset

1= pain, 2=burning, 3=itching, 4=pain and burning, 5=pain and itching, 6=nil

Photos- history of photosensitivity

1=present, 0=absent

Drugs- history of preceding drugs

1=present, 0=absent, 2=not known

Trtmnt- previous specific treattment received

1=yes 0 =no

Steroid- mode of steroid therapy

1=oral, 2=intravenous, 3=pulse only, 4=oral +pulse, 5=intramuscular,

6=oral+intramuscular

Stddos-maximum equvalent dose of oral prednisolone in per kg body weight.

Stddur-duration of steroid therapy in yrs

Curntstd – current steroid dose (equivalent per day dose in mg)

Azoran-treated with azathioprine

1=yes 0-no

Azrndos-dose of azoran in mg /day.

Azrndur - duration of treatment with azathioprine inyrs

Cyclophosph - treated with cyclophosphamide,

1=yes, 0=no

Cyclodos - dose of cyclophosphamide in mg/day

Cyclodur- duration of treatment cyclophosphamideinyrs

Mmf - treated with Mycophenolatemofetil,

1=yes, 0=no

Mmfdos- dose of mycophenolatemofetil, in mg/day

Mmfdur- duration of MMF in yrs

Mtx - treated with methotrexate,

1=yes, 0=no

Mtxdos- dose of methotrexate in mg/week

Mtxdur- duration of methotrexate in years

Dapsone- treated with dapsone,

1=yes, 0=no

Dapdos- dose of dapsone in mg /day

Dapdur- duration of dapsoneinyrs

Ivig - treated with ivig,

1=yes, 0=no

Rxivig- number of times treated with ivig

Ritux – treated with rituximab,

1=yes, 2=no

Rxritux - number of times received rituximab

Comorb- associated comorbidities,

1=yes, 0=no

DM-diabetes mellitus,

1=yes, 0=no

Htn- hypertension,

1=yes, 0=no

Vit D- hypovitaminosis D,

1=yes, 0=no

Thyroid – hypothyroidism,

1=yes 0=no

Others - othercomorbids not mentioned above,

1=yes, 0=no

Othercomor- specify other comorbids _____

Famhist- family h/o pemphigus present,

1=yes, 0=no

Activity - whether in remission or activity

1=activity, 2=remission

Skin - skin is involved,

1=yes, 0=no

Face - face is involved,

1=yes, 0=no

Upperl - upper limbs are involved,

1=yes, 0=no

Lowerl- lower limbs are involved

1=yes, 0=no

Trunk - trunk is involved

1=yes, 0=no

Flex - flexures are involved,

1=yes, 0=no

Genital - genital and perineal region involved

1=yes, 0=no

Bullae - vesicles and bullae present

1=present, 0=absent

Erosion - crusted or moist erosions

1=present, 0=absent

Vegatns - vegetative lesions

1=present, 0=absent

Macules – postinflammatoryhyperpigmented macules

1=present, 0=absent

Acanthoma -acanthoma type of lesion

1=present, 0=absent

Scalp - scalp involvement

1= present, 0= absent

Nails - nails have paronychia

1=present, 0=absent

Mucosa - mucosal involvement is present

1= present, 0=absent

Oral - oral mucosal involvement

1= present, 0=absent

Eye - conjunctival involvement

1=present 0= absent

Nose - nasal mucosal involvement

1= present, 2=absent

Anogenital- anogenital mucosal involvement

1=present 0= absent

Nikolsk - nikolksy sign

1=positive, 0=negative, 2=not done

Bss - bulla spread sign

1=positive, 0=negative, 2= not done

Skinact – activity score in skin (0-120)

Skindam- damage score in skin (0 -13)

Slpscr– scalp score ( 0-10 )

Mucosscr - mucosal score ( 0-120)

PDAI - total PDAI score (0-263)

Tzanck - Tzanck smear

0=negative, 1=acantholytic cell positive, 2=not done

Biopsy- biopsy from skin/mucosal lesion

1=pemphigus vulgaris, 2=pemphigusfoliaceus, 3= not characteristic, 4=not done

DIFiG - Ig type on direct immunofluoresence

1=IgG lowerthird of epidermis, 2=IgG lowerhalf, 3=IgG entire epidermis, 4=IgA, 5= IgG basement membrane, 6=negative, 7=not done

DIFc3 - complement deposit on direct immunofluorescence;

1=c3 lower third, 2=c3lower half of epidermis, 3=c3 entire epidermis 4=c3basal layers, 5=nil 6=not done

dsg1- anti-desmoglein1 levels

dsg3- anti-desmoglein3 levels

IgG4 - serum IgG4 levels

HLADR1- DRB1 1st allele

HLADR2- DRB1 2nd allele

HLADQ3 - DQB1 1st allele

HLADQ4- DQB1 2nd allele

# MASTER CHART

| sno | hosp | age | gend | place | visit | duran | onst | symp | photos | drugs | trtmnt | steroid | stddos | stddur | curntstd |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| V1 | 644014f | 46 | 2 | 1 | 2 | 1.5 | 1 | 5 | 0 | 0 | 1 | 1 | 1 | 1 | 40 |
| v2 | 641638f | 16 | 1 | 4 | 2 | 0.5 | 1 | 6 | 0 | 0 | 1 | 1 | 1.75 | 0.4 | 30 |
| v3 | 585536d | 42 | 1 | 7 | 2 | 6 | 1 | 5 | 0 | 0 | 1 | 1 | 1.5 | 5 | 20 |
| V4 | 708153F | 21 | 2 | 1 | 2 | 1 | 1 | 5 | 0 | 0 | 1 | 1 | 0.3 | 1 | 12.5 |
| V5 | 414211D | 52 | 2 | 1 | 2 | 4.5 | 1 | 1 | 0 | 0 | 1 | 1 | 0.5 | 4 | 2.5 |
| V6 | 228513F | 17 | 2 | 1 | 2 | 2.5 | 1 | 1 | 0 | 0 | 1 | 1 | 0.75 | 2 |  |
| V7 | 704111D | 48 | 2 | 1 | 2 | 3 | 2 | 6 | 1 | 0 | 1 | 4 | 2 | 3 | 1.25 |
| V8 | 645738F | 42 | 1 | 2 | 2 | 5 | 1 | 1 | 1 | 0 | 1 | 4 | 1.3 |  | 25 |
| V9 | 060980F | 45 | 1 | 4 | 2 | 2.5 | 1 | 2 | 0 | 0 | 1 | 1 | 1 | 1.5 | 2.5 |
| V10 | 993076D | 58 | 2 | 3 | 2 | 4 | 3 | 1 | 0 | 0 | 1 | 1 | 0.75 | 4 | 2.5 |
| V11 | 236360F | 12 | 1 | 1 | 1 | 0.5 | 1 | 3 | 0 | 0 | 1 | 1 | 1 | 0.33 | 10 |
| V12 | 772495F | 25 | 1 | 4 | 1 | 2 | 1 | 1 | 0 | 0 | 0 |  |  |  |  |
| V13 | 187349F | 63 | 2 | 8 | 2 | 3 | 2 | 3 | 0 | 0 | 1 | 1 | 1 | 2 | 5 |
| V14 | 782767F | 52 | 2 | 4 | 1 | 10 | 1 | 3 | 1 | 0 | 1 | 4 |  | 10 | 5 |
| V15 | 212061D | 47 | 2 | 1 | 2 | 8 | 1 | 1 | 0 | 0 | 1 | 1 | 1.2 | 7 | 0 |
| V16 | 764284F | 28 | 2 | 1 | 2 | 1.25 | 1 | 4 | 0 | 0 | 1 | 1 | 1 | 0.03 | 50 |
| V17 | 261374F | 26 | 2 | 1 | 2 | 2 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 2 | 0 |
| V18 | 301696F | 49 | 2 | 7 | 2 | 2 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 2 | 2.5 |
| V19 | 184977D | 47 | 1 | 8 | 2 | 6 | 1 | 2 | 0 | 0 | 1 | 1 | 0.6 | 6 | 5 |
| V20 | 375701F | 30 | 2 | 1 | 2 | 1.5 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1.33 | 5 |
| V21 | 787130F | 50 | 1 | 2 | 1 | 0.5 | 3 | 1 | 0 | 0 | 1 | 4 | 1 | 0.41 |  |
| v22 | 732519d | 43 | 2 | 4 | 2 | 5 | 1 | 2 | 0 | 0 | 1 | 1 | 1 | 4 | 5 |
| v23 | 967097d | 55 | 2 | 1 | 2 | 3 | 1 | 3 | 0 | 0 | 1 | 1 | 1 | 2.5 | 5 |
| v24 | 115614f | 46 | 2 | 1 | 2 | 2.5 | 1 | 1 | 0 | 0 | 1 | 1 | 0.75 | 2 | 5 |
| v25 | 803971f | 35 | 2 | 4 | 1 | 0.3 | 1 | 1 | 0 | 0 | 1 | 1 | 0.3 | 0.16 | 20 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| v26 | 826499f | 38 | 1 | 1 | 1 | 4 | 1 | 1 | 0 | 0 | 1 | 4 | 0.5 | 3 | 0 |
| v27 | 498371f | 61 | 2 | 1 | 2 | 1 | 2 | 6 | 0 | 0 | 1 | 1 | 0.5 | 1 | 5 |
| v28 | 831251f | 30 | 1 | 2 | 1 | 0.16 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0.12 | 60 |
| v29 | 314619f | 43 | 1 | 8 | 2 | 1.5 | 1 | 4 | 0 | 0 | 1 | 1 | 1 | 1 | 12.5 |
| v30 | 969123c | 52 | 2 | 1 | 2 | 7 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 7 | 7.5 |
| v31 | 635185d | 31 | 1 | 4 | 2 | 5 | 1 | 1 | 0 | 0 | 1 | 1 | 1.5 | 4 | 20 |
| v32 | 270080d | 29 | 2 | 1 | 2 | 2 | 1 | 4 | 0 | 0 | 1 | 1 | 0.8 | 1 | |
| v33 | 996621d | 39 | 2 | 1 | 2 | 5 | 1 | 1 | 0 | 0 | 1 | 1 | 0.75 | 4 | 7.5 |
| v34 | 342669f | 70 | 1 | 2 | 2 | 2 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1.5 | 7.5 |
| v35 | 709274d | 31 | 2 | 1 | 2 | 4 | 2 | 5 | 0 | 0 | 1 | 1 | 1 | 4 | 2.5 |
| v36 | 855030d | 54 | 1 | 4 | 2 | 3 | 1 | 5 | 0 | 0 | 1 | 1 | 1 | 3 | 5 |
| v37 | 847541f | 49 | 1 | 4 | 1 | 3 | 1 | 2 | 0 | 0 | 1 | 1 | 1 | 2 | 20 |
| v38 | 653505f | 60 | 1 | 5 | 2 | 1 | 1 | 1 | 0 | 0 | 1 | 5 | 1 | 0.66 | 5 |
| v39 | 399599d | 66 | 2 | 6 | 2 | 15 | 1 | 1 | 0 | 0 | 1 | 1 | 0.5 | 15 | 40 |
| v40 | 449434f | 19 | 2 | 4 | 2 | 3 | 1 | | 0 | 0 | 1 | 1 | 1 | 2.5 | 5 |
| v41 | 639189d | 50 | 1 | 2 | 2 | 4 | 3 | 1 | 0 | 0 | 1 | 1 | 1 | 3 | 7.5 |
| v42 | 841906f | 29 | 2 | 4 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 2 | 0.66 | 40 |
| v43 | 862487f | 56 | 2 | 3 | 1 | 1 | 1 | 2 | 0 | 0 | 0 | | | | |
| v44 | 871274f | 24 | 1 | 8 | 1 | 0.25 | 1 | 5 | 1 | 0 | 0 | | | | |
| v45 | 875867f | 32 | 2 | 2 | 1 | 0.83 | 1 | 4 | 0 | 0 | 1 | 1 | 0.6 | 0.5 | 5 |
| v46 | 885044f | 22 | 2 | 1 | 1 | 2 | 2 | | 1 | 0 | 1 | 1 | 0.6 | 2 | 10 |
| v47 | 885129f | 39 | 2 | 5 | 1 | 1 | 1 | 2 | 0 | 0 | 1 | 1 | 0.75 | 0.66 | 60 |
| v48 | 010927g | 58 | 1 | 7 | 1 | 0.41 | 1 | 1 | 0 | 0 | 1 | 4 | 0.5 | 0.25 | |
| v49 | 024603g | 44 | 1 | 8 | 1 | 0.5 | 1 | 1 | 1 | 0 | 1 | 4 | 0.5 | 0.33 | 10 |
| v50 | 031237g | 42 | 2 | 4 | 1 | 3 | 2 | 3 | 0 | 0 | 1 | 1 | 1 | 2 | 5 |
| v51 | 421333d | 57 | 1 | 1 | 2 | 4.5 | 1 | 2 | 0 | 0 | 1 | 1 | 1 | 4 | 10 |
| v52 | 088942f | 66 | 2 | 5 | 2 | 5 | 1 | 4 | 0 | 0 | 1 | 1 | 1 | 4 | 5 |
| f1 | 710291d | 32 | 1 | 1 | 2 | 3.5 | 1 | 3 | 0 | 0 | 1 | 1 | 0.6 | 3.5 | 5 |
| f2 | 676577d | 68 | 1 | 1 | 2 | 5 | 3 | 6 | 0 | 0 | 1 | 1 | 1 | 3 | 5 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| f3 | 788034f | 26 | 1 | 6 | 1 | 1 | 2 | 3 | 1 | 0 | 1 | 1 | 0.7 | 0.8 | 5 |
| f4 | 285308f | 27 | 1 | 4 | 2 | 3.5 | 2 | 3 | 0 | 0 | 1 | 1 | 2 | 3 | 10 |
| f5 | 809482f | 32 | 2 | 4 | 1 | 0.5 | 1 | 1 | 1 | 0 | 1 | 4 | 0.8 | 0.33 | 40 |
| f6 | 606336f | 54 | 1 | 4 | 2 | 3 | 2 | 3 | 0 | 0 | 1 | 1 | 1 | 3 | 12.5 |
| f7 | 831598f | 46 | 2 | 2 | 1 | 0.25 | 2 | 1 | 0 | 0 | 1 | 2 | 1 | 0.16 | 50 |
| f8 | 853709f | 35 | 1 | 8 | 1 | 0.33 | 3 | 3 | 0 | 0 | 1 | 1 | 0.5 | 0.25 | 5 |
| f9 | 847257d | 57 | 1 | 4 | 2 | 4 | 3 | 3 | 0 | 0 | 1 | 4 | 1 | 3.5 | 10 |
| f10 | 733943d | 63 | 1 | 1 | 2 | 6 | 2 | 3 | 0 | 0 | 1 | 1 | 1 | 5 | 5 |
| f12 | 749835f | 58 | 1 | 1 | 1 | 0.18 | 3 | 3 | 0 | 0 | 0 | | | | |
| f13 | 768241f | 45 | 1 | 1 | 1 | 0.16 | 2 | 5 | 0 | 0 | 1 | 1 | 0.2 | 0.08 | 5 |
| f14 | 914077f | 47 | 2 | 1 | 1 | 3 | 1 | 3 | 0 | 0 | 1 | 4 | 0.6 | 2.5 | 0 |
| f15 | 015076g | 42 | 1 | 3 | 1 | 0.6 | 1 | 6 | 0 | 0 | 1 | 1 | 0.3 | 0.5 | 5 |
| f16 | 020317g | 37 | 1 | 8 | 1 | 4 | 2 | 6 | 0 | 0 | 1 | 1 | 0.6 | 3 | 0 |
| f17 | 799031f | 66 | 1 | 6 | 1 | 0.66 | 3 | 3 | 0 | 0 | 1 | 1 | 1 | 0.5 | 0 |
| f18 | 774819f | 60 | 1 | 4 | 1 | 4 | 3 | 3 | 0 | 0 | 1 | 4 | 0.2 | 0.25 | 20 |
| f19 | 031057g | 46 | 2 | 4 | 1 | 4 | 2 | 2 | 0 | 0 | 1 | 4 | 0.6 | 4 | |
| v53 | 406207f | 37 | 2 | 8 | 2 | 1.3 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1.3 | 5 |
| v54 | 017668g | 46 | 1 | 4 | 1 | 2 | 2 | 5 | 0 | 0 | 1 | 1 | 1.2 | 2 | 20 |

| sno | azoran | azrndos | azrndur | cyclophosp | cyclodos | cyclodur | mmf | mmfdos | mmfdur | mtx | mtxdos | mtxdur | dapsone | dapdos | dapdur |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| V1 | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | |
| v2 | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | |
| v3 | 1 | 50 | 3 | 0 | | | 0 | | | 0 | | | 0 | | |
| V4 | 1 | 50 | 0.5 | 0 | | | 0 | | | 0 | | | 1 | 100 | 0.16 |
| V5 | 1 | 50 | 3 | 0 | | | 0 | | | 0 | | | 0 | | |
| V6 | 1 | 50 | 0.5 | 0 | | | 0 | | | 0 | | | 0 | | |
| V7 | 1 | 50 | 0.2 | 0 | | | 1 | 1000 | 0.75 | 0 | | | 0 | | |
| V8 | 1 | 50 | 1.25 | 0 | | | 1 | 1500 | 0.08 | 0 | | | 0 | | |
| V9 | 1 | 75 | 1 | 0 | | | 0 | | | 0 | | | 0 | | |
| V10 | 1 | 100 | 4 | 0 | | | 0 | | | 0 | | | 0 | | |
| V11 | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | |
| V12 | | | | | | | | | | | | | | | |
| V13 | 1 | 50 | 2 | 0 | | | 0 | | | 0 | | | 0 | | |
| V14 | 0 | | | 0 | | | 0 | | | 0 | | | 1 | 100 | 0.2 |
| V15 | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | |
| V16 | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | |
| V17 | 1 | 50 | 0.66 | 0 | | | 0 | | | 0 | | | 0 | | |
| V18 | 1 | 50 | 0.83 | 1 | 50 | 0.02 | 0 | | | 0 | | | 0 | | |
| V19 | 1 | 100 | 5 | 1 | 50 | 0.41 | 0 | | | 0 | | | 0 | | |
| V20 | 1 | 50 | 0.33 | 0 | | | 0 | | | 0 | | | 0 | | |
| V21 | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | |
| v22 | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | |
| v23 | 1 | 50 | 2 | 0 | | | 0 | | | 0 | | | 0 | | |
| v24 | 1 | 25 | 1.5 | 0 | | | 0 | | | 0 | | | 0 | | |
| v25 | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | |
| v26 | 0 | | | 1 | 50 | 2.5 | 0 | | | 0 | | | 0 | | |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| v27 | 0 | | | 0 | | | 0 | | | 0 | | | 1 | 100 | 0.12 |
| v28 | 0 | | | 0 | | | 0 | | | 0 | | | 1 | 100 | 0.12 |
| v29 | 1 | 25 | 1 | 0 | | | 0 | | | 0 | | | 0 | | |
| v30 | 1 | 50 | 1 | 0 | | | 0 | | | 0 | | | 0 | | |
| v31 | 1 | 50 | 1.5 | 0 | | | 0 | | | 0 | | | 1 | 100 | 1 |
| v32 | | | | | | | | | | | | | | | |
| v33 | 1 | 50 | 0.12 | 1 | 75 | 0.5 | 0 | | | 0 | | | 1 | 100 | 0.16 |
| v34 | 1 | 50 | 0.16 | 0 | | | 0 | | | 0 | | | 0 | | |
| v35 | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | |
| v36 | 1 | 50 | 2 | 0 | | | 0 | | | 0 | | | 0 | | |
| v37 | 1 | 50 | 0.66 | 1 | 50 | 0.5 | 0 | | | 0 | | | 0 | | |
| v38 | 1 | 100 | 0.66 | 0 | | | 0 | | | 0 | | | 0 | | |
| v39 | 0 | | | 0 | | | 0 | | | 1 | 5 | 2 | 0 | | |
| v40 | 1 | 50 | 2 | 0 | | | 0 | | | 0 | | | 0 | | |
| v41 | 1 | 100 | 0.25 | 0 | | | 0 | | | 0 | | | 0 | | |
| v42 | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | |
| v43 | | | | | | | | | | | | | | | |
| v44 | | | | | | | | | | | | | | | |
| v45 | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | |
| v46 | 0 | | | 1 | 50 | 0.33 | 0 | | | 1 | 7.5 | 0.12 | 1 | 100 | 2 |
| v47 | 1 | 100 | 0.25 | 0 | | | 0 | | | 0 | | | 0 | | |
| v48 | 0 | | | 1 | 50 | 0.16 | 0 | | | 0 | | | 0 | | |
| v49 | 0 | | | 1 | 50 | 0.03 | 0 | | | 0 | | | 0 | | |
| v50 | 1 | 100 | 1.5 | 0 | | | 0 | | | 0 | | | 0 | | |
| v51 | | | | | | | | | | | | | | | |
| v52 | 1 | 50 | 0.6 | 0 | | | 0 | | | 0 | | | 0 | | |
| f1 | 1 | 50 | 3 | 0 | | | 0 | | | 0 | | | 0 | | |
| f2 | 1 | 50 | 0.25 | 1 | 200 | 0.33 | 0 | | | 1 | 7.5 | 0.5 | 1 | 50 | 0.25 |
| f3 | 0 | | | 0 | | | 0 | | | 1 | 7.5 | 0.08 | 0 | | |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| f4 | 1 | 50 | 0.25 | 0 | | | 0 | | | 0 | | | 0 | | |
| f5 | 1 | 50 | 0.33 | 0 | | | 0 | | | 0 | | | 0 | | |
| f6 | 0 | | | 0 | | | 0 | | | 1 | 15 | 1.5 | 1 | 50 | 1 |
| f7 | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | |
| f8 | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | |
| f9 | 1 | 50 | 0.5 | 0 | | | 0 | | | 0 | | | 0 | | |
| f10 | 0 | | | 0 | | | 0 | | | 0 | | | 1 | 100 | 4 |
| f12 | | | | | | | | | | | | | | | |
| f13 | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | |
| f14 | 0 | | | 1 | 50 | 2 | 0 | | | 0 | | | 0 | | |
| f15 | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | |
| f16 | 1 | 150 | 2 | 1 | 100 | 0.25 | 0 | | | 0 | | | 0 | | |
| f17 | 0 | | | 0 | | | 0 | | | 0 | | | 0 | | |
| f18 | 1 | 50 | 0.03 | 1 | 100 | 0.08 | 0 | | | 0 | | | 1 | 100 | 0.08 |
| f19 | 0 | | | 1 | 50 | 4 | 0 | | | 0 | | | 0 | | |
| v53 | 1 | 50 | 0.5 | 0 | | | 0 | | | 0 | | | 0 | | |
| v54 | 1 | 50 | 1 | 0 | | | 0 | | | 0 | | | 0 | | |

| sno | ivig | rxivig | ritux | rxritux | comorb | dm | htn | vitd | thyroid | others | othercomor | famhist | activity | skin | face |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| V1 | 0 | | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | asthma | 0 | 1 | 1 | 1 |
| v2 | 0 | | 0 | 0 | 0 | | | | | | | 0 | 1 | 1 | 0 |
| v3 | 0 | | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | osteoporosis | 0 | 1 | 1 | 1 |
| V4 | 0 | | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | atopy | 0 | 1 | 0 | |
| V5 | 0 | | 0 | | 1 | 1 | 1 | 0 | 0 | 0 | | 0 | 1 | 1 | 0 |
| V6 | 0 | | 0 | | 0 | | | | | | | 0 | 1 | 1 | 0 |
| V7 | 1 | 1 | 0 | | 1 | 0 | 0 | 0 | 1 | 0 | | 0 | 2 | | |
| V8 | 0 | | 0 | | 1 | 0 | 0 | 0 | 0 | 1 | hiv | 0 | 1 | 1 | 1 |
| V9 | 0 | | 0 | | 0 | | | | | | | 0 | 2 | | |
| V10 | 0 | | 0 | | 1 | 1 | 1 | 1 | 0 | 0 | | 0 | 2 | | |
| V11 | 0 | | 0 | | 1 | 0 | 0 | 1 | 0 | 0 | | 0 | 1 | 1 | 1 |
| V12 | | | | | 0 | | | | | | | 0 | 1 | 0 | |
| V13 | 0 | | 0 | | 1 | 1 | 1 | 1 | 0 | 1 | osteoporosis | 0 | 2 | | |
| V14 | 0 | | 0 | | 1 | 0 | 1 | 0 | 0 | 0 | | 0 | 2 | | |
| V15 | 0 | | 0 | | 1 | 1 | 1 | 0 | 0 | 0 | | 0 | 1 | 1 | 0 |
| V16 | 0 | | 0 | | 0 | | | | | | | 0 | 1 | 0 | |
| V17 | 0 | | 0 | | 1 | 0 | 0 | 1 | 0 | 1 | | 0 | 1 | 1 | 1 |
| V18 | 0 | | 0 | | 1 | 0 | 1 | 1 | 0 | 0 | | 0 | 2 | | |
| V19 | 0 | | 0 | | 1 | 0 | 0 | 1 | 0 | 0 | | 0 | 1 | 0 | |
| V20 | 0 | | 0 | | 0 | | | | | | | 0 | 1 | 0 | |
| V21 | 0 | | 0 | | 0 | | | | | | | 0 | 1 | 1 | 1 |
| v22 | 0 | | 0 | | 0 | | | | | | | 0 | 1 | 0 | |
| v23 | 0 | | 0 | | 1 | 1 | 1 | 0 | 0 | 0 | | 0 | 2 | | |
| v24 | 0 | | 0 | | 0 | | | | | | | 0 | 2 | | |
| v25 | 0 | | 0 | | 1 | 0 | 0 | 1 | 0 | 0 | | 0 | 1 | 1 | 0 |
| v26 | 0 | | 0 | | 1 | 0 | 0 | 1 | 0 | 0 | | 0 | 1 | 1 | 1 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| v27 | 0 | | 0 | | 1 | 0 | 0 | 0 | 0 | 1 | nhl retroperitonealfibro | 0 | 2 | | |
| v28 | 0 | | 0 | | 1 | 0 | 0 | 1 | 0 | 0 | | 0 | 1 | 1 | 1 |
| v29 | 0 | | 0 | | 1 | 0 | 1 | 1 | 0 | 1 | | 0 | 1 | 0 | |
| v30 | 0 | | 0 | | 0 | | | | | | | 0 | 1 | 0 | |
| v31 | 0 | | 0 | | 1 | 0 | 0 | 0 | 0 | 1 | osteopenia | 0 | 1 | 1 | 1 |
| v32 | | | | | | | | | | | | | | | |
| v33 | 0 | | 0 | | 0 | | | | | | | 0 | 1 | 1 | 1 |
| v34 | 0 | | 0 | | 1 | 1 | 1 | 1 | 0 | 0 | | 0 | 1 | 1 | 1 |
| v35 | 0 | | 0 | | 1 | 0 | 0 | 1 | 0 | 0 | | 0 | 1 | 0 | |
| v36 | 0 | | 0 | | 1 | 0 | 1 | 0 | 0 | 0 | | 0 | 1 | 1 | 0 |
| v37 | 0 | | 0 | | 1 | 0 | 0 | 0 | 0 | 1 | osteopenia | 0 | 1 | 1 | 1 |
| v38 | 0 | | 0 | | 1 | 0 | 0 | 1 | 0 | 0 | | 0 | 1 | 1 | 1 |
| v39 | 0 | | 0 | | 1 | 1 | 1 | 1 | 0 | 0 | | 0 | 1 | 1 | 0 |
| v40 | 0 | | 0 | | 0 | | | | | | | 0 | 2 | | |
| v41 | 0 | | 0 | | 1 | 1 | 1 | 0 | 0 | | | 0 | 1 | 1 | 0 |
| v42 | 0 | | 0 | | 1 | 1 | 0 | 1 | 0 | 1 | osteopenia | 0 | 1 | 1 | 1 |
| v43 | | | | | 1 | 1 | 0 | 0 | 0 | 1 | osteoporosis dyslipid | 0 | 1 | 1 | 0 |
| v44 | | | | | 1 | 0 | 0 | 1 | 0 | 0 | | 0 | 1 | 1 | 0 |
| v45 | 0 | | 0 | | 0 | | | | | | | 0 | 1 | 1 | 1 |
| v46 | 0 | | 0 | | 1 | 0 | 0 | 0 | 0 | 0 | | 0 | 1 | 1 | 1 |
| v47 | 0 | | 0 | | 1 | 1 | 1 | 1 | 1 | 0 | | 0 | 1 | 1 | 0 |
| v48 | 0 | | 0 | | 1 | 0 | 0 | 1 | 0 | 0 | | 0 | 1 | 1 | 1 |
| v49 | 0 | | 0 | | 1 | 0 | 0 | 1 | 0 | 0 | | 0 | 1 | 1 | 0 |
| v50 | 0 | | 0 | | 1 | 0 | 0 | 1 | 0 | 0 | | 0 | 1 | 1 | 1 |
| v51 | | | | | 1 | 1 | 1 | 0 | 0 | 1 | hepatitis c | 0 | 1 | 1 | 1 |
| v52 | 0 | | 0 | | 1 | 1 | 0 | 1 | 0 | 1 | osteopenia | 0 | 1 | 1 | 0 |
| f1 | 0 | | 0 | | 0 | | | | | | | 0 | 1 | 1 | 1 |
| f2 | 0 | | 0 | | 0 | | | | | | | 0 | 1 | 1 | 1 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| f3 | 0 | | 0 | | 0 | | | | | | | 0 | 1 | 1 | 1 |
| f4 | 0 | | 0 | | 1 | 1 | 0 | 0 | 0 | 1 | Osteopenia | 0 | 1 | 1 | 1 |
| f5 | 0 | | 0 | | 1 | 0 | 0 | 1 | 0 | 0 | | 0 | 1 | 1 | 1 |
| f6 | 0 | | 0 | | 1 | 1 | 1 | 0 | 0 | 0 | | 0 | 1 | 1 | 0 |
| f7 | 0 | | 0 | | 0 | | | | | | | 0 | 1 | 1 | 1 |
| f8 | 0 | | 0 | | 1 | 1 | 0 | 0 | 0 | 0 | | 0 | 1 | 1 | 1 |
| f9 | 0 | | 0 | | 1 | 1 | 0 | 0 | 0 | 1 | chronic liver disease | 0 | 1 | 1 | 0 |
| f10 | 0 | | 0 | | 1 | 0 | 1 | 0 | 0 | 1 | adjustment disorder | 0 | 1 | 1 | 0 |
| f12 | | | | | 1 | 1 | 1 | 0 | 0 | 0 | | 0 | 1 | 1 | 1 |
| f13 | 0 | | 0 | | 1 | 1 | 0 | 1 | 0 | 0 | | 0 | 1 | 1 | 1 |
| f14 | 0 | | 0 | | 1 | 1 | 0 | 0 | 1 | 0 | | 0 | 1 | 1 | 1 |
| f15 | 0 | | 0 | | 0 | | | | | | | 0 | 1 | 1 | 0 |
| f16 | 0 | | 0 | | 1 | 1 | 0 | 0 | 0 | 1 | tuberculosis | 0 | 1 | 1 | 1 |
| f17 | 0 | | 0 | | 1 | 1 | 0 | 1 | 0 | 0 | | 0 | 1 | 1 | 1 |
| f18 | 0 | | 0 | | 1 | 1 | 0 | 1 | 0 | 0 | | 0 | 1 | 1 | 1 |
| f19 | 0 | | 0 | | 1 | 1 | 0 | 1 | 0 | 1 | osteoporosis | 0 | 1 | 1 | 0 |
| v53 | 0 | | 0 | | 0 | | | | | | | 0 | 1 | 0 | |
| v54 | 0 | | 0 | | 1 | 0 | 0 | 1 | 0 | 1 | osteoporosis | 0 | 1 | 1 | 1 |

| sno | upperl | lowerl | trunk | flex | genital | bullae | erosion | vegatns | macules | acanthoma | scalp | nails | mucosa | oral | eye | nose | anogenital |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| V1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 |
| v2 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 |
| v3 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 |
| V4 | | | | | | | | | | | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| V5 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| V6 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| V7 | | | | | | | | | | | | | | | | | |
| V8 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 |
| V9 | | | | | | | | | | | | | | | | | |
| V10 | | | | | | | | | | | | | | | | | |
| V11 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | | | | |
| V12 | | | | | | | | | | | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| V13 | | | | | | | | | | | | | | | | | |
| V14 | | | | | | | | | | | | | | | | | |
| V15 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| V16 | | | | | | | | | | | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| V17 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| V18 | | | | | | | | | | | | | | | | | |
| V19 | | | | | | | | | | | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| V20 | | | | | | | | | | | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| V21 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 |
| v22 | | | | | | | | | | | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| v23 | | | | | | | | | | | | | | | | | |
| v24 | | | | | | | | | | | | | | | | | |
| v25 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 |
| v26 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 |
| v27 | | | | | | | | | | | | | | | | | |
| v28 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| v29 | | | | | | | | | | | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| v30 | | | | | | | | | | | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| v31 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 |
| v32 | | | | | | | | | | | | | | | | | |
| v33 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| v34 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | | | | |
| v35 | | | | | | | | | | | 1 | 0 | 1 | 1 | 0 | 0 | 0 |
| v36 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| v37 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 |
| v38 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| v39 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | | | | |
| v40 | | | | | | | | | | | | | | | | | |
| v41 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | | | | |
| v42 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | | | | |
| v43 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| v44 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| v45 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 |
| v46 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| v47 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| v48 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| v49 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 |
| v50 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | | | | |
| v51 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | | | | |
| v52 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| f1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | | | | |
| f2 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | | | | |
| f3 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | | | | |
| f4 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | | | | |
| f5 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | | | | |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| f6 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | | | | |
| f7 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | | | | |
| f8 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | | | | |
| f9 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | | | | |
| f10 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | | | | |
| f12 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | | | | |
| f13 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | | | | |
| f14 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | | | | |
| f15 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | | | | |
| f16 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | | | | |
| f17 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | | | | |
| f18 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | | | | |
| f19 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | | | | |
| v53 | | | | | | | | | | | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| v54 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | | | | |

| sno | nikolsk | bss | skinact | skindam | slpscr | mucosscr | PDAI | tzanck | biopsy | difig | difc3 | dsg1 | dsg3 | igg4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| V1 | 0 | 1 | 50 | 0 | 3 | 1 | 54 | 1 | 1 | 2 | 2 | 210 | 156 | 1150 |
| v2 | 0 | 1 | 43 | 4 | 1 | 12 | 60 | 2 | 1 | 2 | 2 | 147 | 173 | |
| v3 | 0 | 2 | 3 | 6 | 0 | 12 | 21 | 2 | 4 | 7 | 6 | 135 | 161 | 786 |
| V4 | 2 | 2 | 0 | 2 | 0 | 5 | 7 | 0 | 1 | 3 | 5 | 85 | 155 | |
| V5 | 2 | 2 | 0 | 4 | 0 | 1 | 5 | 2 | 1 | 2 | 2 | 2 | 122 | 1365 |
| V6 | 0 | 2 | 0 | 2 | 0 | 15 | 17 | 1 | 1 | 2 | 4 | 13 | 160 | 760 |
| V7 | | | | | | | | 2 | 1 | 2 | 4 | 6.3 | 7.1 | |
| V8 | 0 | 0 | 10 | 3 | 10 | 25 | 48 | 2 | 2 | 7 | 6 | 49 | 143 | 748 |
| V9 | | | | | | | | 2 | 4 | 7 | 6 | 7.6 | 170 | |
| V10 | | | | | | | | 2 | 4 | 7 | 6 | 5.5 | 211 | |
| V11 | 0 | 1 | 16 | 3 | 3 | 0 | 22 | 2 | 3 | 1 | 5 | 43 | 202 | 1299 |
| V12 | 2 | 2 | 0 | 0 | 0 | 3 | 3 | 2 | 1 | 1 | 1 | 12.2 | 176 | 1033 |
| V13 | | | | | | | | 1 | 1 | 1 | 5 | 2 | 2 | |
| V14 | | | | | | | | 2 | 4 | 7 | 6 | 15.2 | 122 | |
| V15 | 2 | 2 | 0 | 3 | 0 | 1 | 4 | 2 | 1 | 2 | 2 | 1.5 | 99 | 704 |
| V16 | 2 | 2 | 0 | 0 | 0 | 5 | 5 | 1 | 4 | 6 | 5 | 8 | 166 | 1898 |
| V17 | 0 | 0 | 19 | 6 | 3 | 18 | 46 | 2 | 1 | 1 | 5 | 182 | 185 | 923 |
| V18 | | | | | | | | 2 | 1 | 1 | 5 | 2 | 67.4 | |
| V19 | 2 | 2 | 0 | 0 | 0 | 3 | 3 | 2 | 1 | 3 | 1 | 2 | 143 | 760 |
| V20 | 2 | 2 | 0 | 0 | 0 | 1 | 1 | 2 | 4 | 2 | 5 | 4.5 | 187 | 713 |
| V21 | 1 | 1 | 31 | 6 | 4 | 4 | 45 | 2 | 1 | 2 | 2 | 190 | 202 | 1829 |
| v22 | 2 | 2 | 0 | 0 | 0 | 5 | 5 | 2 | 1 | 2 | 4 | 15 | 4.2 | 1829 |
| v23 | | | | | | | | 2 | 4 | 2 | 5 | | | |
| v24 | | | | | | | | 1 | 1 | 6 | 5 | 3.8 | 4.8 | |
| v25 | 0 | 0 | 10 | 4 | 3 | 57 | 74 | 1 | 1 | 1 | 1 | 148 | 169 | 605 |
| v26 | 1 | 2 | 3 | 1 | 3 | 11 | 18 | 1 | 2 | 5 | 4 | 2.3 | 195 | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| v27 | | | | | | | | 1 | 3 | 2 | 5 | 2 | 16.4 | |
| v28 | 1 | 1 | 49 | 8 | 1 | 17 | 75 | 0 | 1 | 3 | 4 | 72 | 144 | 601 |
| v29 | 2 | 2 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 4 | 2 | 14 | 596 |
| v30 | 2 | 2 | 0 | 0 | 0 | 7 | 7 | 1 | 1 | 3 | 4 | 31.3 | 194 | 1557 |
| v31 | 1 | 1 | 26 | 7 | 1 | 5 | 39 | 1 | 1 | 2 | 4 | 256 | 200 | 1463 |
| v32 | | | | | | | | | 1 | 2 | 2 | | | |
| v33 | 2 | 2 | 0 | 3 | 0 | 3 | 6 | 0 | 1 | 1 | 5 | 5.6 | 79 | 715 |
| v34 | | | | | | | | 0 | 1 | 1 | 4 | 161 | 144 | 1426 |
| v35 | 2 | 2 | 0 | 0 | 1 | 3 | 4 | 1 | 1 | 2 | 5 | 29 | 198 | |
| v36 | 0 | 0 | 0 | 1 | 0 | 2 | 3 | 2 | 1 | 6 | 2 | 2 | 66 | 672 |
| v37 | 0 | 0 | 4 | 2 | 3 | 2 | 11 | 2 | 1 | 2 | 2 | 260 | 252 | 3199 |
| v38 | 0 | 0 | 4 | 3 | 0 | 3 | 10 | 2 | 4 | 2 | 2 | 2.2 | 249 | 1201 |
| v39 | 0 | 0 | 2 | 0 | 0 | 0 | 2 | 2 | 1 | 1 | 1 | 2 | 2 | 698 |
| v40 | | | | | | | | 1 | 1 | 2 | 4 | 2 | 11 | 758 |
| v41 | 0 | 0 | 0 | 1 | 1 | 0 | 2 | 2 | 1 | 6 | 5 | 68.5 | 76.3 | 605 |
| v42 | 0 | 0 | 11 | 8 | 1 | 0 | 20 | 1 | 1 | 1 | 1 | 14.7 | 243 | 605 |
| v43 | 0 | 0 | 2 | 2 | 0 | 3 | 7 | 2 | 1 | 6 | 5 | 151 | 121 | 772 |
| v44 | 1 | 1 | 10 | 2 | 0 | 36 | 48 | 2 | 1 | 1 | 1 | 3.06 | 248 | 1964 |
| v45 | 0 | 0 | 13 | 9 | 3 | 25 | 50 | 1 | 1 | 2 | 2 | 9.2 | 257 | 1749 |
| v46 | 1 | 1 | 40 | 11 | 4 | 2 | 57 | 1 | 1 | 2 | 2 | 180 | 256 | 881 |
| v47 | 0 | 2 | 10 | 7 | 3 | 12 | 32 | 1 | 3 | 2 | 4 | 66 | 250 | 646 |
| v48 | 0 | 1 | 10 | 7 | 3 | 15 | 35 | 0 | 4 | 2 | 1 | 127 | 261 | 813 |
| v49 | 0 | 0 | 20 | 4 | 0 | 51 | 75 | 0 | 1 | 2 | 2 | 37 | 221 | 902 |
| v50 | 0 | 0 | 2 | 3 | 0 | 0 | 5 | 2 | 4 | 7 | 6 | 51 | 249 | 602 |
| v51 | 0 | 2 | 9 | 4 | 10 | 0 | 23 | 1 | 1 | 1 | 1 | 267 | 250 | 2224 |
| v52 | 0 | 0 | 13 | 7 | 2 | 5 | 27 | 1 | 1 | 1 | 1 | | | |
| f1 | 0 | 2 | 2 | 1 | 0 | 0 | 3 | 1 | 2 | 2 | 4 | 146 | 2 | 605 |
| f2 | 0 | 2 | 5 | 9 | 1 | 0 | 15 | 1 | 2 | 3 | 5 | 200 | 2 | 754 |
| f3 | 1 | 1 | 32 | 6 | 4 | | 41 | 1 | 4 | 3 | 3 | 166 | 86 | 714 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| f4 | 0 | 2 | 3 | 3 | 0 | 0 | 6 | 0 | 4 | 3 | 5 | 163 | 2.1 | 1898 |
| f5 | 1 | 1 | 95 | 0 | 10 | 0 | 105 | 1 | 4 | 3 | 1 | 234 | 117 | 7378 |
| f6 | 0 | 2 | 0 | 3 | 0 | 0 | 3 | 0 | 2 | 3 | 3 | 26.4 | 2 | 602 |
| f7 | 1 | 1 | 85 | 0 | 3 | 0 | 88 | 0 | 4 | 2 | 2 | 223 | 163 | 617 |
| f8 | 0 | 1 | 11 | 6 | 1 | 0 | 18 | 0 | 2 | 2 | 2 | 291 | 2.2 | 892 |
| f9 | 0 | 0 | 5 | 6 | 1 | 0 | 12 | 2 | 2 | 3 | 3 | 130 | 2.5 | 1263 |
| f10 | 0 | 0 | 17 | 6 | 0 | 0 | 23 | 1 | 2 | 2 | 2 | 204 | 32 | 605 |
| f12 | 1 | 1 | 18 | 2 | 10 | 0 | 30 | 1 | 2 | 3 | 3 | 150 | 29 | 605 |
| f13 | 1 | 1 | 26 | 0 | 4 | 0 | 30 | 1 | 4 | 3 | 3 | 234 | 94 | 1518 |
| f14 | 1 | 1 | 70 | 7 | 4 | 0 | 81 | 1 | 2 | 3 | 3 | 242 | 3 | 605 |
| f15 | 0 | 0 | 2 | 2 | 0 | 0 | 4 | 2 | 2 | 3 | 5 | 136 | 2 | 1475 |
| f16 | 1 | 2 | 22 | 10 | 10 | 0 | 42 | 1 | 4 | 2 | 2 | 231 | 0 | 605 |
| f17 | 1 | 2 | 37 | 0 | 4 | 0 | 41 | 0 | 2 | 3 | 3 | 1 | 1 | 1610 |
| f18 | 1 | 2 | 30 | 7 | 10 | 0 | 47 | 1 | 2 | 3 | 2 | 234 | 33 | 2881 |
| f19 | 0 | 2 | 11 | 6 | 3 | 0 | 20 | 2 | 2 | 2 | 2 | | | 990 |
| v53 | 2 | 2 | 0 | 0 | 0 | 2 | 2 | 2 | 1 | 7 | 6 | 2 | 19.4 | 894 |
| v54 | 0 | 0 | 3 | 5 | 3 | 0 | 11 | 2 | 4 | 2 | 2 | 5.3 | 94 | 1007 |

| sno | hospno | DRB1a | DQB1a | DRB1b | DQB1b | s no. | hospno | DRB1a | DQB1a | DRB1a | DQB1b |
|---|---|---|---|---|---|---|---|---|---|---|---|
| V1 | 644014f | 11 | 03 | 14 | 05 | C1 | 431684f | 09 | 03 | 10 | 05 |
| V2 | 641638f | 04 | 03 | 14 | 05 | C2 | 369860f | 13 | 06 | 03 | 02 |
| V3 | 585536d | 10 | 05 | 14 | 05 | C3 | 340733f | 15 | 06 | 15 | 06 |
| V4 | 708153F | 08 | 03 | 14 | 05 | C4 | 404040f | 12 | 03 | 14 | 05 |
| V5 | 414211D | 14 | 05 | 15 | 06 | C5 | 144058f | 04 | 02 | 07 | 03 |
| V6 | 228513F | 07 | 03 | 14 | 05 | C6 | 776406d | 07 | 02 | 11 | 03 |
| V7 | 704111D | 07 | 02 | 14 | 05 | C7 | 448672f | 03 | 02 | 14 | 05 |
| V8 | 645738F | 04 | 03 | 14 | 05 | C8 | 402692f | 15 | 06 | 15 | 06 |
| V9 | 060980F | 07 | 03 | 14 | 05 | C9 | 441411f | 14 | 05 | 13 | 06 |
| V10 | 993076D | 04 | 03 | 14 | 05 | C10 | 426140f | 07 | 03 | 10 | 05 |
| V11 | 236360F | 14 | 05 | 14 | 05 | C11 | 457016f | 07 | 03 | 11 | 02 |
| V12 | 772495F | 14 | 05 | 14 | 05 | C12 | 495996f | 08 | 03 | 14 | 05 |
| V13 | 187349F | 04 | 03 | 07 | 03 | C13 | 409926f | 13 | 05 | 15 | 06 |
| V14 | 782767F | 12 | 03 | 14 | 05 | C14 | 624827f | 14 | 05 | 14 | 05 |
| V15 | 212061D | 14 | 05 | 14 | 05 | C15 | 465678f | 15 | 06 | 15 | 06 |
| V16 | 764284F | 14 | 05 | 15 | 06 | C16 | 491652f | 04 | 03 | 13 | 06 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| V17 | 261374F | 14 | 05 | 14 | 05 | C17 | 492596f | 12 | 03 | 14 | 05 |
| V18 | 301696F | 04 | 03 | 14 | 05 | C18 | 931164c | 15 | 06 | 11 | 03 |
| V19 | 184977D | 04 | 03 | 10 | 05 | C19 | 049725c | 07 | 02 | 13 | 06 |
| V20 | 375701F | 07 | 03 | 14 | 05 | C20 | 206513d | 07 | 02 | 07 | 02 |
| V21 | 787130F | 04 | 03 | 14 | 05 | C21 | 675880f | 07 | 03 | 12 | 03 |
| V22 | 732519d | 12 | 03 | 14 | 05 | C22 | 650207f | 01 | 03 | 04 | 05 |
| V23 | 967097d | 04 | 03 | 14 | 05 | C23 | 418943f | 15 | 06 | 14 | 05 |
| V24 | 115614f | 04 | 03 | 14 | 05 | C24 | 694842c | 13 | 05 | 14 | 06 |
| V25 | 803971f | 07 | 03 | 14 | 05 | C25 | 623729f | 14 | 05 | 07 | 02 |
| V26 | 826499f | 13 | 05 | 14 | 06 | C26 | 714904f | 04 | 03 | 12 | 04 |
| V27 | 498371f | 03 | 02 | 15 | 06 | C27 | 647570f | 07 | 03 | 14 | 05 |
| V28 | 831251f | 14 | 05 | 15 | 06 | C28 | 729495f | 04 | 03 | 15 | 06 |
| V29 | 314619f | 08 | 04 | 14 | 05 | C29 | 750412f | 11 | 03 | 12 | 03 |
| V30 | 969123c | 14 | 05 | 14 | 05 | C30 | 206907d | 10 | 05 | 15 | 06 |
| V31 | 635185d | 11 | 03 | 14 | 05 | C31 | 769727f | 12 | 03 | 11 | 03 |
| V33 | 996621d | 14 | 05 | 14 | 05 | C32 | 722988f | 15 | 05 | 15 | 06 |
| V35 | 709274d | 04 | 04 | 14 | 05 | C33 | 784197f | 04 | 03 | 11 | 03 |
| V36 | 855030d | 14 | 05 | 15 | 05 | C34 | 700890f | 01 | 03 | 07 | 05 |
| V37 | 847541f | 07 | 02 | 14 | 05 | C35 | 766321f | 11 | 03 | 14 | 05 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| V38 | 653505f | 14 | 05 | 15 | 06 | C36 | 632549f | 11 | 03 | 15 | 06 |
| V40 | 449434f | 12 | 03 | 14 | 05 | C37 | 605972f | 14 | 05 | 15 | 06 |
| V41 | 639189d | 14 | 05 | 14 | 05 | C38 | 037534f | 15 | 06 | 10 | 05 |
| V42 | 841906f | 14 | 03 | 14 | 05 | C39 | 499704f | 15 | 05 | 14 | 05 |
| V43 | 862487f | 14 | 05 | 16 | 05 | C40 | 792128f | 01 | 05 | 10 | 05 |
| V44 | 871274f | 14 | 05 | 15 | 05 | C41 | 616181f | 14 | 05 | 15 | 05 |
| V45 | 875867f | 08 | 04 | 14 | 05 | C42 | 753831f | 14 | 05 | 15 | 06 |
| V46 | 885044f | 08 | 03 | 14 | 05 | C43 | 670039f | 04 | 03 | 12 | 03 |
| V48 | 010927g | 14 | 03 | 14 | 05 | C44 | 801004f | 12 | 03 | 15 | 06 |
| V49 | 024603g | 04 | 03 | 14 | 05 | C45 | 783347f | 07 | 03 | 12 | 03 |
| V50 | 031237f | 07 | 02 | 14 | 05 | C46 | 819431f | 07 | 02 | 14 | 05 |
| V51 | 421333d | 04 | 03 | 14 | 05 | C47 | 492570f | 11 | 03 | 11 | 03 |
| V52 | 088942f | 13 | 06 | 14 | 06 | C48 | 743932f | 07 | 03 | 07 | 02 |
| V53 | 406207f | 14 | 05 | 15 | 06 | C49 | 023624f | 12 | 03 | 04 | 03 |
| V54 | 017668g | 04 | 03 | 14 | 05 | C50 | 053531f | 07 | 02 | 07 | 02 |